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Identifying constraints to increasing yield potential of spring barley

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Declaration

I hereby declare that this thesis has been composed by me and that the work presented herein is my own, unless otherwise stated. This work has not been submitted for any other degree or professional qualification.

Shane Kennedy

Lay Summary

Ireland achieved the second highest yield of barley in the world during the first decade of this millennium. Over 85 % of the Irish crop is spring-sown barley, commonly known as spring barley. The aim of research reported in this thesis was to establish what factors determine the yield of spring barley in a productive environment like Ireland. This information can be used by growers to improve the efficiency of cereal production and by the scientific community to identify ways of further increasing yield.

Field experiments were carried out at several locations in Ireland from 2011 to 2013. These crops yielded on average 8.5 tonnes of grain per hectare. The yield of any area of crop has two main components: the number of grains in that area and the average weight of those individual grains. The number of grains had the greatest influence on yield and was most influenced by the number of ears, or seed heads, at harvest. Crops with larger and heavier shoots earlier in the season were most likely to produce high ear numbers and hence high grain numbers. When very high ear number crops were produced, the number of grains in each ear was reduced. An optimum of approximately 1000 ears per m² at harvest was identified.

Through photosynthesis, crops convert the energy from light to chemical energy, which can be stored in the dry matter of the plant tissue. Over half of this is stored as starch in the grains. Evidence from this and other work suggests that barley has the potential to create more dry matter than it has grains to store it. This suggests that output (yield) can be increased by managing or breeding for crops that have more grains or grains with a greater storage capacity. Field experiments where light availability to crops was manipulated using shade netting gave an insight into how these yield components are determined. Results suggest that grain number and grain storage capacity may both be determined during a relatively short period of growth during stem extension.

Calculations indicate a maximum yield potential of over 12 tonnes of grain per hectare under Irish conditions. It is likely that increases in growth during stem extension will be required to achieve this.

Abstract

The literature suggests that grain number largely determines and as such limits yield in barley. Many of the reported studies were conducted in relatively low yielding environments and it is unclear if grain number is also a limiting factor in high yield potential climates. Nor is it known with certainty what physiological or morphological traits must be targeted in order to increase grain number. There may be a degree of trade-off between yield components whereby grain number is adjusted according to resource availability to the plant, either pre- or post-anthesis, in a way that ensures consistently well-filled grains at harvest. If mechanisms exist for adjusting grain numbers or grain storage capacity after anthesis to match assimilate availability, this may place limits on how far yield can be increased without increasing post-anthesis assimilate production. In order to determine the scope for increasing the yield potential of barley a more thorough understanding of the potential trade-offs between grain number, grain storage capacity and post-anthesis assimilate supply is required. The aim of research reported in this thesis was to establish what determines the yield of spring barley in Ireland and to investigate the timing and possible mechanisms involved in regulating grain number and grain storage capacity in relation to the supply of photoassimilates.

Field experiments were carried out on spring barley (*Hordeum vulgare* L., cv. Quench) at several locations in Ireland from 2011 to 2013. A sub-set of experiments involving destructive sampling and in-field assessments on plots managed as per current best farm practice gathered crop growth, development, and yield component data across sites and seasons in order to establish what determines yield under typical crop production conditions. Separate experiments artificially manipulated the source:sink ratio of plots via shading and seed rate treatments to investigate in more detail the mechanisms determining grain number and grain weight and any potential trade-off between the two components.

Grain number accounted for most of the variation in yield across 9 site/seasons of crops managed as per current best practice in Ireland ($P < 0.001$; $R^2 = 0.84$) while grain weight remained relatively conserved. Ear number accounted for most of the variation in grain number ($P = 0.002$; $R^2 = 0.75$) and ear number itself was largely

determined by shoot survival from an early season peak through to harvest ($P < 0.001$; $R = 0.96$). Shoot size and weight at the beginning of stem extension had the largest influence on shoot survival.

Shading treatments were used to test whether there was a mechanism for adjusting grain numbers after anthesis to match the availability of assimilate for grain filling. Substantial post-anthesis reductions in assimilate supply during grain filling in 2011 and 2012 did not significantly reduce grain number ($P > 0.05$). A small reduction in grain number (8%) was found in response to shading for a two week period early post-anthesis in 2013, however this was likely a reduction in grain set in shoots or spikelets that reached anthesis after the treatment was imposed rather than a post-anthesis abortion or down-regulation of grain number. Percentage light interception by well managed (unshaded) canopies shortly after anthesis was generally greater than 93% across several sites and seasons, therefore increasing grain numbers to increase sink capacity would likely be associated with an unavoidable decrease in the amount of light intercepted per grain during the early grain development period. However, experiments showed that grain weight at harvest was neither reduced nor increased in response to variations in light interception during this period of endosperm development ($P > 0.05$), because soluble sugar concentrations in the grain were maintained at the expense of storage reserve deposition in the stems.

Results suggest that grain number and grain storage capacity may both be determined pre-anthesis resulting in a trade-off during stem extension whereby grain numbers are adjusted in a way that helps conserve grain weight. A strong negative relationship between ear number and grain number per ear ($P < 0.001$; $R^2 = 0.81$) across two sites of seed rate experiments in 2013 resulted in a plateau in overall grain number of approximately 18,000 grains m^{-2} suggesting that there may be a limit to how many grains can be established in a given environment; this was achieved with an ear number of approximately 1000 ears m^{-2} .

Yield potential for Irish conditions was estimated at 12.29 t ha^{-1} at 85% dry matter based on estimates of potential assimilate supply during grain filling; with a grain number of 26,481 m^{-2} required to utilise this. These estimates are both 44% higher than the mean yield and grain number achieved in crops managed as per current best

farm practice. Once high potential ear numbers are secured ($> 1000 \text{ m}^{-2}$), breaking the negative relationship between ear number and grain number per ear may hold the key to further increasing grain number and hence yield potential. Increasing assimilate production and partitioning to ears during stem extension, either through increases in the duration of stem extension or solar radiation use efficiency, may enable larger grain numbers to be produced whilst maintaining or increasing individual grain storage capacity and deposition of stem storage reserves. Water and nutrient availability, as well as susceptibility to lodging may present further limitations to yield in the future.

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List of Abbreviations

ANOVA analysis of variance

CK Cork experimental site

CW Carlow experimental site

DAFM Department of Agriculture, Food and the Marine

ESC ethanol soluble carbohydrates

EWSC ethanol and water soluble carbohydrates

GAD green area duration post-anthesis

GAI green area index

GDD growing degree days

GS growth (developmental) stage

GSC grain storage capacity

HI harvest index

KK Kilkenny experimental site

MGW mean grain weight

MS main stem

PAR photosynthetically active radiation

RUE radiation use efficiency

T1 first tiller emerged after the main stem

T2 second tiller emerged after the main stem

TTC 2,3,5 Triphenyltetrazolium Chloride

WX Wexford experimental site

General Introduction

In terms of the world's most important crops by production quantity, barley (*Hordeum vulgare* L.) is ranked fourth amongst the cereals after maize, rice and wheat, and eleventh overall (Newton et al., 2011). Barley is one of the oldest domesticated crops and in recent times 55-60% of the world crop has been used for animal feed, 30-40% for malting and brewing, about 5% for seed, and 2-3% for direct human consumption (Ullrich, 2011). Perhaps the presence of a fibrous hull on the barley grain led to the prominence of maize, rice and wheat as human and non-ruminant food sources (Ullrich, 2011). Nonetheless, future prospects for barley production are bright given its stress tolerant reputation, wide geographic range, variety of end uses and potential contribution to future human nutrition (Jenkins, 1985; Newton et al., 2011).

The world population is increasing by 200,000 per day (Anon., 2011c). It has already broken the 7 billion mark and is estimated to reach over 10 billion by 2100 as a result of better health care services and the greater number of people in the reproductive age group (Anon., 2011c). Declines in fertility may slow the increase but these figures present a challenge in terms of food security. Anon. (2012b) predicts a 60% increase in demand for agricultural production by 2050 and demand for cereals is projected to rise to 3 billion tonnes – a 43% increase from today's 2.1 billion tonnes (Anon., 2009). Optimising the performance of crops in areas of high yield potential is one possible approach to help meet the future increases in food demand whilst minimising global land use change. Furthermore, maximising grain productivity is key to optimising the economic performance of agriculture, and to reducing the greenhouse gas costs of production. Achieving consistently high yields without causing environmental damage will require 'ecological intensification' of cereal production (Cassman, 1999).

Newton et al. (2011) describe yield potential as yield of adapted varieties in a given location where water and nutrients are non-limiting and weeds, pests and disease are absent. Actual yield is the realised portion of yield potential achieved by growers in the field. Yield potential increases through breeding are believed to be responsible for between one third and half of the total actual yield gains achieved in the past 50

years while improved agronomy (mainly the increased use of N fertilizer) is largely responsible for the rest (Abeledo et al., 2003; Bell et al., 1995; Slafer et al., 2005). Recent studies have shown that crop yields in European countries are increasing at a slower rate and that agronomic factors related to environmental policy and economic return rather than a lack of genetic improvement are responsible (Berry and Spink, 2006; Brisson et al., 2010; Finger, 2010; Lillemo et al., 2010; Peltonen-Sainio et al., 2009). This may be due to the marginal cost-benefit of additional inputs as farm yields approach the yield potential ceiling (van Wart et al., 2013). Yields of major cereal crops appear to plateau at 70–90% of estimated yield potential (Cassman, 1999; Cassman et al., 2003; Grassini et al., 2011; Grassini et al., 2009; Lobell et al., 2009). Abeledo et al. (2003) confirm that trends in actual and potential yields of barley and other crops tend to be parallel. Improvements in actual cereal yields may well depend upon further increases in yield potential, despite the fact that there is a gap between the two (Slafer et al., 2005). Also, future economic and environmental constraints on agronomic inputs may mean that future increases in actual yield may only be achieved by growers if yield potential increases (Abeledo et al., 2003). Further, the current and predicted rates of increase in yield potential are less than the expected increase in demand (Cassman, 1999; Hall and Richards, 2013). Accelerated improvement in genetic yield potential is required in order to meet demand and avoid encroachment into natural landscapes or the over-intensification of current agro-ecosystems (Reynolds et al., 2009). Whilst narrowing the yield gap through improved targeting of agronomic efforts is important it must be accompanied by an increase in yield potential (Newton et al., 2011; Slafer et al., 2005).

Increased food demand is likely to narrow the gap between potential and realized yields in the most productive environments; global food security in the near future will depend on rapid advances in understanding the physiological basis of crop yield potential (Cassman, 1999). High yields are an obvious requirement of any breeding programme (Newton et al., 2011). Breeding for improved productivity has been tremendously successful in the past (Bulman et al., 1993; Grausgruber et al., 2002; Slafer et al., 2005), but needs to be more efficient in the future (Slafer et al., 2005). Marker assisted selection and genomic selection techniques can identify genes responsible for specific biotic and abiotic stresses and produce better performing

cultivars (Newton et al., 2011; Slafer et al., 2005) however yield is a complex trait to single out (Cassman, 1999) and is strongly influenced by environment. Contributions from molecular technology for improved yield potential will depend upon improved knowledge of the physiological mechanisms of yield determination (Fischer, 2008; Slafer et al., 2005). An increased understanding of the basic physiology of yield will be required to both drive genetic yield potential advances and further close the yield gap particularly if resource-use efficiency becomes a major target for breeders (Newton et al., 2011).

Irish agriculture is predominantly grass based with cereal production accounting for 6.7% (177,000 ha) of the total farmed area (average 2000 to 2009 (Anon., 2011b)). Of this area devoted to cereals, 60% is barley (Anon., 2011b). There are several possible reasons why barley occupies a large percentage of the cropped area in Ireland. Barley has always been the dominant crop especially in the early 1980's and farming businesses have traditionally been family owned and run and handed down through the generations and it may be that the tradition of planting barley has been handed down also. Given the grass based nature of Irish agriculture and the need to house livestock during the wet winter months there is a strong local market for barley grain (and straw) as animal feed. Further, a strong malting market has boosted the production of barley.

Ireland contributed 0.8% of the global barley tonnage during the period 2000-2009 (Anon., 2011a). Although a relatively small player worldwide, Ireland achieves the second highest yield of barley in the world at 6.6 t ha⁻¹ (Anon., 2011a) (based on data from 2000-2009). This coupled with the fact that over 85% of the Irish barley crop is spring-sown barley (Anon., 2011b), which is inherently lower yielding than winter-sown, indicates the high yield potential of the temperate maritime Irish climate. This region of high yield potential provides an excellent opportunity to test the limitations to same.

Looking at historical yield data for the Irish barley crop, it appears that yields are continuing to increase but that perhaps the rate of increase is slowing (Table 0.1). This may be a consequence of increased input costs, a changing regulatory environment where inputs are limited, or a change in the growing environment of the

crop. On the other hand, it could also be that the gap between genetic yield potential and field yields is narrowing. In general however, it is difficult to ascertain whether yields are in fact plateauing because of the large amount of year-on-year yield variation (Figure 0.1).

Table 0.1. Barley (includes spring- and winter-sown) yield increase on a decade by decade basis in Ireland (Anon., 2011a).

Time Period	Average Yield (t ha ⁻¹)	% increase on previous decade
1961-1970	3.6	
1971-1980	4.2	+ 20%
1981-1990	5.4	+ 28%
1991-2000	6.1	+ 12%
2001-2010	6.6	+ 9%

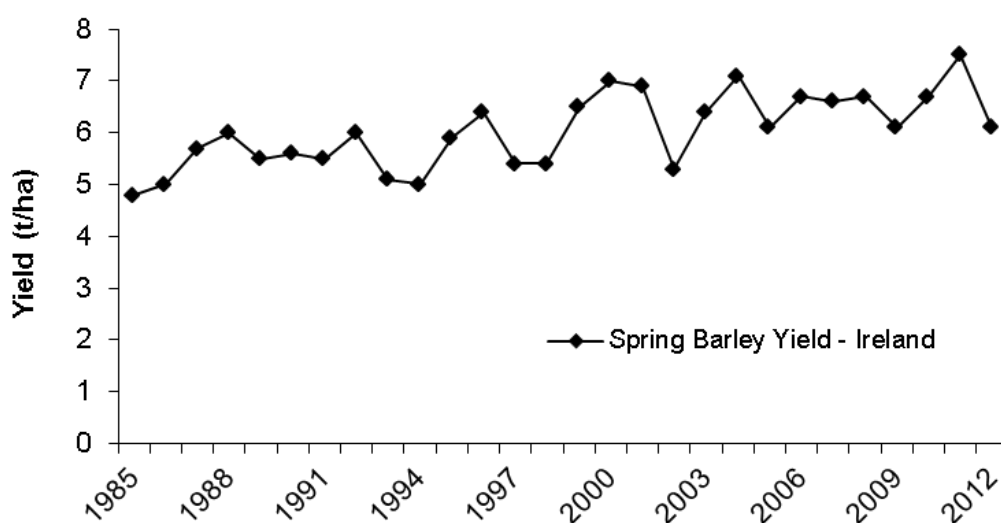


Figure 0.1. Year-on-year variability in Irish spring barley yields (Anon., 2012a).

To assess the limitations to yield imposed by site, seasons, husbandry practices and cultivars it is first necessary to have an understanding of the principles governing the response of crops to their environment. This project aims to improve understanding of the basic physiology of yield in the Irish context by monitoring the growth, development and yield of barley crops across sites and seasons. Understanding site and season variation will help identify what is responsible for high yields and whether there is scope for growers to further increase yield with the current genetic material available through improved agronomic practices e.g. better targeting of inputs. Concurrent, more targeted, field experimentation aims to better understand the mechanisms and processes governing yield thus identifying possible routes for increasing yield potential.

Chapter 1 **Background**

1.1 Physiological determination of yield

Yield potential can be expressed as a function of the amount of photosynthetically active radiation intercepted by the canopy (RI), the efficiency with which that energy is converted into dry matter (radiation use efficiency RUE) and the fraction of dry matter partitioned into harvested components (harvest index HI) (Newton et al., 2011; Reynolds et al., 2005)

$$YP = RI \times RUE \times HI$$

These three components are dynamic and their interaction throughout the season determines yield potential (Reynolds et al., 2005). Increasing yield potential will involve increasing any or all of these component values.

Not all solar radiation is available for photosynthesis – approximately half of it (that which is in the 400-700 nm wavelength range) is photosynthetically active radiation (PAR) (Biscoe et al., 1975c; Hay and Porter, 2006). The proportion of this PAR that can be intercepted by the crop (RI) is affected by canopy size, duration and architecture (Newton et al., 2011). Canopy size is largely determined by the number of shoots and the number and size of leaves (Newton et al., 2011) and is described using a green area index (GAI) which is the ratio of green area per unit ground area. Maximum GAI is reached around anthesis (Ramos et al., 1995) after which point crops begin to senesce. In barley a GAI of 5 can intercept around 95% of incident radiation (Bingham and Topp, 2009). Canopy architecture i.e. leaf angle, has little impact on RI in canopies of adequate GAI (Newton et al., 2011). The greatest scope for increasing RI is to improve canopy establishment early in the season to hasten canopy closure and prolong GAI post-anthesis (Newton et al., 2011; Richards, 2000).

Grain yield per unit area increases in barley and other crops in the past were largely due to increases in biomass partitioning to the grain (HI) but the consensus in the literature is that current genetic material is approaching the upper limit of HI of approximately 0.50 – 0.60 (Cassman, 1999; Hay, 1995; Jenkins, 1985; Miralles and Slafer, 2007; Naylor et al., 1998; Reynolds et al., 2005; Riggs et al., 1981; Slafer et

al., 2005). Further yield potential increases will most likely be achieved by increases in total biomass (Naylor et al., 1998) by focusing on constraints to RUE i.e. combining a high biological yield with a high HI (Riggs et al., 1981).

RUE is expressed as accumulated above ground dry matter per unit of PAR intercepted (g MJ^{-1}). RUE is affected by carbon losses due to respiration and photorespiration – not all of the assimilate produced by photosynthesis is used in the production of new plant tissue (Gallagher et al., 1983). Barley is a C_3 plant and its productivity is thus the result of a balance between CO_2 fixation via photosynthesis and CO_2 loss through dark respiration and photorespiration (Smith et al., 1999). The enzyme Rubisco catalyses the assimilation of CO_2 in the leaf but can also catalyse wasteful oxygenation thus initiating photorespiration (Reynolds et al., 2009). Genetic engineering for an increased specificity for CO_2 over O_2 would reduce the energy expenditure associated with photorespiration and overall increases in levels of the enzyme Rubisco could further increase photosynthetic rate (Reynolds et al., 2009). RUE is also affected by canopy architecture, whereby a more erect leaf habit could theoretically increase RUE by reducing light saturation of the upper leaves of the canopy and allowing light to penetrate the lower leaf layers (Angus et al., 1972; Reynolds et al., 2009; Reynolds et al., 2000). However, evidence in the major grain crops suggest that photosynthetic capacity at anthesis and beyond (source) is not limiting (Bingham et al., 2007a; Dreccer et al., 1997; Richards, 2000; Serrago et al., 2013; Slafer and Savin, 1994) and the yield of barley in a range of environments is considered to be limited by the number of grains available for dry matter accumulation and the capacity of those grains to store dry matter (sink) (Arisnabarreta and Miralles, 2008a; Bingham et al., 2007a; Savin et al., 2006). Such a restricted demand for photosynthate can lead to a feed-back inhibition of photosynthesis (Bingham et al., 2007a) thereby reducing RUE below its potential (Newton et al., 2011).

Many discussions on yield and the grain filling period in cereal production are centered on the source-sink relationship of the crop in question. Source can be defined as the energy captured and converted in the production of carbon assimilates by the plant to fuel growth. Source available for grain growth has two possible

origins - from post-anthesis photosynthesis and from storage reserves in the form of soluble and insoluble carbohydrates built up during the growth period prior to anthesis. These storage reserves are usually in the stem portion of the shoot. The major sink for many plants is the grain. Sink tissues are net importers of assimilates as they are unable to synthesize enough themselves to meet their own demands. Sink capacity can refer to not only the number of grains but also the grain storage capacity (GSC) - the ability of the grains to accumulate dry matter. Improved RUE may be achieved by increasing demand, or sink size, if excess photosynthetic capacity, or source, exists during grain filling (Reynolds et al., 2009; Reynolds et al., 2005; Reynolds et al., 2000). Considering RUE as something that can vary over the crop cycle in response to environment and supply/demand of photosynthate may be more intuitive (Reynolds et al., 2005) and achieving the optimum source-sink balance will increase RUE (Reynolds et al., 2009).

Expressing yield potential as a season long analysis of crop growth and yield formation like this is useful, but it is important to realise that it operates under the limits set by crop development.

1.2 Growth versus development

It is important to outline the differences between crop growth and crop development.

Crop growth, as the term suggests, can be expressed as a change in mass (dry matter) or a change in area (associated with the division and expansion of cells). Through photosynthesis, crops use the energy from the solar radiation they intercept to fix CO₂ and, in the process, convert it to chemical energy stored in organic matter - the dry matter of the plant tissue. Solar radiation drives plant growth.

Crop development may be described as the progress of a crop towards maturity (Gallagher et al., 1983). Crop development can be split into many defined phases by set phenological stages as per the Zadoks decimal scale outlined in Tottman (1987). These stages are commonly known (somewhat contradictorily) as growth stages (GS) and indicate the degree of progress of a crop through its lifecycle. In short- to mid-

length growing seasons such as spring-sown crops, several crop development stages can overlap. The timing of phenological stages, and therefore the duration of the intervening phases, is determined mainly by accumulated temperature (Gallagher et al., 1976; Hay and Porter, 2006). High temperatures shorten the phase between two developmental stages (rapid development) and low temperatures will prolong the phase (slow development). High yielding environments are typically bright and cool – high radiation levels promote good dry matter accumulation and cool temperatures prolong the growing season (Newton et al., 2011)

Photoperiod (day length) and vernalisation also influence development (Ellis et al., 1989) but less so in spring varieties (Gallagher et al., 1975; Hay and Porter, 2006; Kirby and Appleyard, 1984). Day length is a primary factor in inducing plants to develop reproductive structures (Frank and Bauer, 1995) and responses are triggered when night period falls below a critical value. Varieties differ in their response to developmental factors. If photoperiod is sub-optimal for the variety in question then progress towards anthesis will be delayed (Roberts et al., 1988). Long exposure to cold (vernalisation) accelerates flowering in winter cereals (Distelfeld et al., 2009; von Zitzewitz et al., 2005). Vernalisation genes in winter varieties delay the transition from vegetative development to reproductive development (Hay and Porter, 2006; Newton et al., 2011) and as such crops will not progress to anthesis until after the cold winter months have been endured. This is an important trait for winter crops as it prevents frost damage at anthesis and consequent yield loss (Newton et al., 2011). Many barley varieties flower without a vernalisation requirement which is useful for adaption to warmer growing conditions (Sasani et al., 2009) e.g. a spring sowing. Spring barley varieties do not require vernalisation (von Zitzewitz et al., 2005). Also, plants are vernalised in the dark (Sasani et al., 2009) therefore vernalisation is independent of photoperiod.

Because of the strong influence of temperature on crop development, the concept of thermal time ($^{\circ}\text{C}$ days) is often used in physiological analyses of crop growth. Thermal time is the mean daily temperature accumulated above a fixed base temperature (usually 0°C) below which it is assumed no development occurs. Using thermal time allows a more standardised comparison of crop growth across different

sites and seasons. Another useful measure in crop physiology, but one that takes into account both solar radiation and temperature, is photothermal quotient. Assuming that water and nutrients are not limiting, the single variable of photothermal quotient, which is the ratio of the mean daily intercepted radiation and mean daily temperature, can be used as per Estrada-Campuzano et al. (2008) as an indicator or predictor of total biomass or dry matter growth during a given developmental period.

The growth and yield formation of barley is broadly considered in two major crop developmental phases – the first from crop emergence to anthesis where the organs required for assimilation and accumulation of harvestable dry weight are initiated and formed (i.e. the canopy, roots and potential grain sites) and the second from anthesis to maturity where grain development and filling takes place (Nicolas et al., 1985). The pre-anthesis phase can be further sub-divided into vegetative and reproductive phases followed by fertilisation and seed set at anthesis. The crop cycle then moves into a grain development and grain growth phase followed by a ripening phase just prior to harvest (Slafer et al., 2009). The environmental factors mentioned above, along with others (e.g. soil moisture deficit, nutrient stress, plant density) will impact upon development during each phase (Smith et al., 1999)

Following germination in barley (and wheat) the crown of the plant develops just below soil level where the shoot apex becomes active (Kirby and Appleyard, 1984). The apex consists of a meristematic dome – a region of active cell division that initiates primordia which will develop and grow into the various organs of the plant (Kirby and Appleyard, 1984). This initiation occurs in a sequential order and the precise apex development stage is determined by the development of the most advanced primordium (Smith et al., 1999). Descriptive scales of apical development have been developed by Kirby and Appleyard (1984) and Waddington and Cartwright (1983). In the larger context of the Zadoks scale outlined in Tottman (1987), apical development spans the pre-stem extension period. Apical development continues until all of the spikelet primordia are initiated on the embryo ear. This completes prior to stem extension (Kirby and Appleyard, 1984) beyond which primordia simply develop and grow – there is no further initiation. In short- to mid-length growing seasons, e.g. spring-sown crops, the duration of apical development

is usually brief, and as with overall crop development, several apical development stages may overlap (Smith et al., 1999). Figure 1.1 below illustrates important sub-phases of development for a typical spring-sown barley crop in the context of canopy size and the Zadoks decimal scale (Tottman, 1987). Further details on the sub-phases illustrated will be discussed in subsequent sections of this chapter.

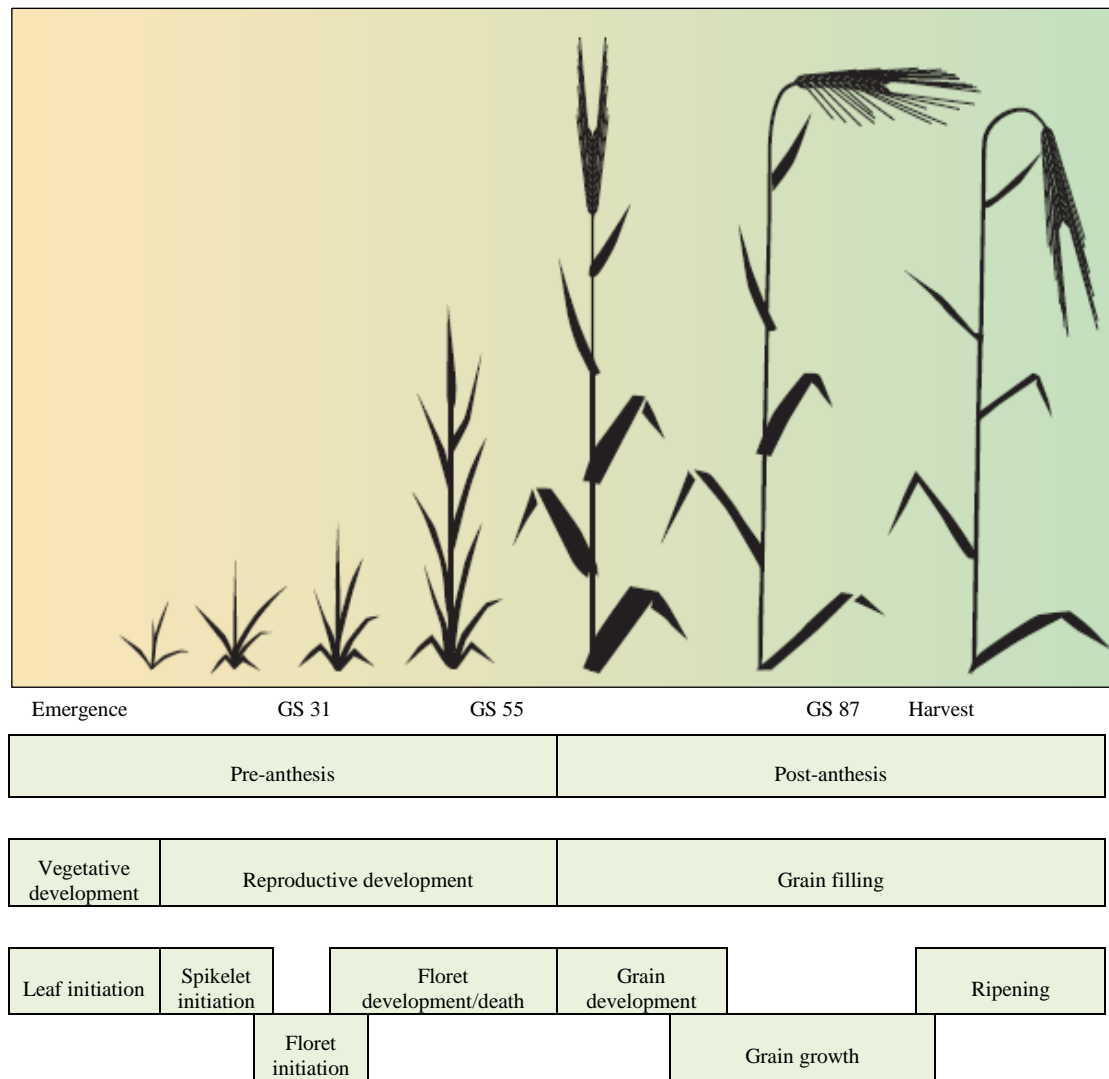


Figure 1.1. Important crop developmental phases in a spring-sown barley lifecycle. Modified from Slafer and Rawson (1994) and Slafer et al. (2009). Illustration portion from Spink et al. (2006). Decimal growth stages (GS) as per the Zadoks decimal scale outlined in Tottman (1987).

1.3 Vegetative development

The vegetative phase of development is one in which the leaves are initiated as primordia consequent of cell division on the shoot apex (Slafer et al., 2009). The primordia are in the form of ridges and will grow to form leaves that eventually emerge from sub-tending leaf sheaths. The embryo in a seed contains a shoot apex and an apical dome with up to four leaf primordia already pre-developed (Kirby, 1977). During vegetative development between eight and fifteen leaf primordia are formed (Kirby and Appleyard, 1984). The first leaf emerges through the coleoptile – a sheath-like structure designed to thrust through the soil during emergence and protect the developing shoot apex (Kirby and Appleyard, 1984). The coleoptile will have elongated from the seed to bring the shoot apex up to a level just below the soil surface. Subsequent leaves emerge from the growing point, each one unfurling from the sheath of the previous one.

Vegetative initiation of leaf primordia continues until the onset of reproductive initiation by which time the maximum number of potential leaves in the main shoot is determined (Slafer et al., 2009). The shoot apex will remain in the vegetative phase until three to six leaves have emerged on the main shoot (Kirby and Appleyard, 1984) at which point it is still at or just below ground level. The cessation of leaf initiation in grain crops normally occurs in response to a photoperiodic signal once vernalisation (if required) has occurred (Hay and Porter, 2006). Winter varieties generally have more leaves than spring varieties because they remain in a vegetative state for longer.

The plastochron and the phyllochron are two relatively constant measures of development relating to leaf initiation and leaf emergence (Slafer et al., 2009). The plastochron ($^{\circ}\text{C}$ days) is the reciprocal of the rate of leaf primordia initiation and the phyllochron ($^{\circ}\text{C}$ days) is the reciprocal of the rate at which the leaves appear. As indicators of development, they are influenced by the factors mentioned in section 1.2, namely temperature and photoperiod.

1.4 Reproductive development

Spikelet primordia, also in the form of ridges on the shoot apex, develop in the region immediately above each leaf primordia ridge (Kirby and Appleyard, 1984). The point at which they become visible is known as ‘double ridge stage’ and marks the beginning of floret initiation and hence reproductive development (Kirby and Appleyard, 1984) despite the fact that the apex is likely to have already initiated half its maximum number of spikelet primordia by this point (Kirby, 1977). A plant at double ridge stage will have four to nine leaves emerged on the main shoot (Kirby and Appleyard, 1984). At this point the shoot apex is about 1 mm long and still at or just below ground level as further leaves continue to emerge. Initiated spikelets differentiate further as other spikelets and parts of the ear continue to be initiated (Kirby and Appleyard, 1984). This continues until the awn primordium stage when the embryo ear has its full complement of spikelet primordia (Kirby, 1977) – the meristematic dome at the tip of the shoot apex now ceases activity and begins to dry-up (Kirby and Appleyard, 1984). The meristematic dome experiences a decline in physical size from a maximum at double-ridge stage and in the run up to its death some of the latter formed spikelet primordia will also die (Kirby, 1977; Kirby and Faris, 1970). At this stage the shoot apex is still at or just below soil level, enclosed by the developing leaves. It takes about 50-65 days for spring crops and 200 days for winter crops in the UK to reach this point (Gallagher et al., 1976; Kirby and Appleyard, 1984).

During the floret initiation phase of apical development a maximum of thirty-five – forty-five spikelet primordia are formed (Gallagher et al., 1976; Kirby, 1977; Kirby and Appleyard, 1984). The initial size and development rate of floral primordia is affected by their position on the shoot apex (Kirby, 1977). The later initiated spikelets develop faster to provide some degree of synchronicity at the end of the floret initiation phase (Kirby, 1977; Kirby and Appleyard, 1984), however there may be some incomplete development and spikelets do differ in size. The first ‘collar’ primordium at the base of the embryo ear is the smallest and spikelet primordia increase in both length and diameter to a maximum at around the centre of the vertical axis of the apex above which spikelet length and diameter decreases towards

the tip (Kirby, 1977). As such, the largest spikelets can be found in the central locations on the embryo ear – a difference which persists until grain maturity (Evers and Millar, 2002; Kirby, 1977).

Floret initiation is followed by floret development as the spikelets develop florets prior to anthesis. The barley ear consists of several spikelets which contain the florets which can upon fertilization develop into grains. The axis of the ear, known as the rachis is usually bilaterally symmetrical with three spikelets at each node (Smith et al., 1999). Each spikelet consists of one floret and two narrow glumes and each floret consists of a lemma, palea, a carpel and three stamens (Smith et al., 1999). Of the three spikelets at each node, only the floret in the central (median) spikelet is potentially fertile in two-row varieties (generally spring varieties) – barley of this type will only have two rows of grains up the length of the ear (Kirby and Appleyard, 1984; Smith et al., 1999; Spink et al., 2006). Six-row varieties (generally winter varieties) can have fertile florets at all three spikelets on each node. Floret development occurs during the stem extension phase of development which brings the developing ear upwards within the stem until it emerges from the ensheathing flag leaf at anthesis (Kirby and Appleyard, 1984; Slafer et al., 2009). During this time the vascular connections to the spikelets develop (Kirby and Rymer, 1974) the stamen (including anthers) are growing to surround the developing carpel (including the stigma, styles and ovule) and the palea and lemma will also grow to ultimately enclose these reproductive organs (Kirby and Appleyard, 1984). At harvest, the lemma and palea remain attached to the caryopsis of barley grains after threshing (Kirby and Appleyard, 1984; Smith et al., 1999).

Stem extension is a phase where there is a large increase in total crop growth rate which is mirrored by a rather ‘abrupt’ increase in length and dry weight of the embryo ear (Kirby, 1977). Competition for assimilate between the ear and other rapidly growing plant organs e.g. the stem, and competition within the ear itself can result in spikelet death (Gallagher et al., 1976). Lesser developed and smaller distal spikelets at the tip and base of the may not be able to compete for insufficient resources and between 20% - 50% either die or do not all fully develop the floral organs necessary for fertilisation at anthesis (Gallagher et al., 1976; Kirby, 1977).

Additionally, florets are particularly sensitive to stress during meiosis (nuclear and cell division in preparation for anthesis) and this can result in sterility or decreased grain set (Kirby and Appleyard, 1984).

When the stem grows, internodes elongate and the nodes become detectable. The last internode (between the uppermost node and ear collar) is known as the peduncle. The 'knots' on the nodes play an important role in directing the growth habit of the crop whereby if lodging occurs, variable rates of growth on opposite sides of a node can return the peduncle and ear to a vertical position (Kirby and Appleyard, 1984).

1.5 Tillering

As plant main stems progress through the primordia initiation phases described in sections 1.3 to 1.4, tillers (side-shoots/branches), emerge from buds differentiated from primordium units on the shoot apex (Kirby, 1977; Kirby and Appleyard, 1984). Primary tiller buds originate in the axils of the basal leaves of the main stem and secondary tiller buds can originate in the axils of the basal leaves of the primary tiller stem (Kirby and Appleyard, 1984). It is also possible for tertiary tillers to grow from similar buds in secondary tillers. Tiller buds develop their own independent shoot apex and meristematic dome (Kirby and Appleyard, 1984). A prophyll encloses the tiller shoot apex similar to how the coleoptile encloses the main stem shoot apex and the first leaf will emerge from the prophyll (Kirby and Appleyard, 1984). From here the tiller shoot apex progresses through the same initiation phases of development as the main stem shoot apex, albeit slightly delayed. Later tillers produce fewer leaf and spikelet primordia (Gallagher et al., 1976) and this helps synchronise the development of all the shoots in a crop to an extent. Not all tiller buds will grow to become tillers therefore tiller bud initiation is not indicative of tiller production. The onset of tillering or 'branching' is approximately three phyllochrons after seedling emergence (i.e. the first tiller after the main stem emerges when leaf three is visible) and plants can produce many tillers but as growth resources become limiting some will die (Gallagher et al., 1976; Hay and Kirby, 1991; Slafer et al., 2009).

The traditional tillering pattern involves a rapid increase in the first few weeks post-emergence reaching a maximum around the beginning of stem extension as the crop moves into the floral initiation stage of development (del Moral et al., 1984). This is generally followed by a period of tiller death which largely occurs during stem extension (del Moral et al., 1984; Gallagher et al., 1975) and then remains stable until harvest (del Moral et al., 1984; Gallagher et al., 1975; Slafer et al., 2009). A flush of late tillering is possible in response to a rainfall event for example, but the contribution of these late tillers to yield is thought to be negligible (Kirby, 1967). Tiller death occurs due to the intense competition for resources during stem extension as discussed in section 1.4. Prior to emergence, tiller buds are very dependent on the photosynthetic activity of the source leaf (Fletcher and Dale, 1974). Tillers during their early growth and development continue to rely heavily on the main stem or parent stem for photoassimilates (Kirby and Jones, 1977; Smith et al., 1999). As tiller buds grow and produce their own leaf area they become less dependent on the parent tiller (Lauer and Simmons, 1988) and can even provide photoassimilate to the main shoot (Elalaoui et al., 1992; Lauer and Simmons, 1988). However tillers compete with the main shoot for a limited supply of resources e.g. light and nitrogen (Kirby and Jones, 1977). Around stem extension, main shoot photoassimilate translocation shifts away from the tillers and towards the main stem itself (Lauer and Simmons, 1985) and this along with the propensity for over production (Kirby and Faris, 1972) can also result in tiller death (Kirby, 1977). This tiller death can help with the synchrony and convergence of development in the crop but the initiation and growth of leafy, non-ear bearing tillers could also be regarded as wasteful (Kirby and Jones, 1977; Thorne and Wood, 1987). It is possible for some of the carbon and nitrogen assimilated by non-surviving shoots to move into the rest of the plant as they die (Thorne, 1962; Thorne and Wood, 1987) but the production of large amounts of non-surviving shoots has been shown to ultimately be detrimental to yield potential in wheat especially in drought situations where dry matter in dying shoots may be less easily remobilized (Berry et al., 2003).

In a spring-sown barley crop (cv. Proctor) of standard seed rate in the UK a maximum of 1500 shoots m^{-2} (including main stems and tillers) was produced but only 927 shoots m^{-2} bore ears that reached anthesis (Gallagher et al., 1976). Tiller

mortality can vary with cultivar and environment (Kirby and Riggs, 1978; Simmons et al., 1982; Thorne, 1962) – % survival rates of 68.3% to 37.4% (from maximum tiller number to harvest tiller number) have been recorded in field studies of several varieties and types of barley in contrasting Mediterranean environments (del Moral and del Moral, 1995). Tiller survival is generally higher for earlier produced tillers (Davidson and Chevalier, 1990; Gallagher et al., 1976; Kirby and Riggs, 1978). Survival rates of shoots in UK grown spring-barley crops were highest for the main stem, followed by the tiller borne out of the axil of the first true leaf, the coleoptile node tiller, and the tiller borne out of the axil of the second true leaf, in that order (Cannell, 1969). In wheat, Thorne and Wood (1988), identify the main stem and the tillers in the axils of the first two leaves as accounting for most of the 566 ears m⁻² present at harvest in UK field experiments in the following proportions: main stem 56%; first tiller 26%; second tiller 16%.

Tillering is an important compensatory mechanism in yield determination (Cannell, 1969), and, as it is a crop developmental phase, is influenced by the environmental factors mentioned in section 1.2. Further influences on tiller production and survival will be discussed in section 1.10.

1.6 Anthesis, fertilisation and seed set

Anthesis, commonly known as flowering, lasts only a few minutes in barley florets and generally occurs in all ears in the crop within a few days (Kirby and Appleyard, 1984). It marks the end of floret development and the start of grain development. Anthesis occurs when pollen that fertilises the ovule is released from the anthers. In open-flowering varieties, lodicules swell to push apart the lemma and palea and the anthers can be seen protruding out of the floret. Spring varieties tend to be closed flowering where the anthers are less visible and often anthesis occurs before or whilst the ear is emerging from the flag leaf sheath (Evers and Millar, 2002; Kirby and Appleyard, 1984). Pollination occurs when the pollen falls on the feathery stigma. Following pollination, a pollen tube develops through which male nuclei enter the

embryo sac and fertilisation occurs when nuclei fusion gives rise to the embryo and endosperm (Evers and Millar, 2002).

1.7 Grain development and grain filling

The barley grain can be divided into three components: the husk, the embryo, and the endosperm (Fabian et al., 2011). The latter, comprising approximately 80% of final grain dry weight, is the most economically important (Evers and Millar, 2002). The embryo, which will comprise about 15% of the final grain dry weight (Kirby and Appleyard, 1984), will include root and shoot meristems and several leaf initials at maturity (Gubatz and Shewry, 2011). The size of the endosperm and embryo are also important factors influencing the early vigour (but not germination) of seedlings (Fabian et al., 2011). The endosperm consists of starch granules embedded in a matrix of storage proteins (Evers and Millar, 2002). Also included as part of the endosperm is a surrounding layer of aleurone cells have relatively high concentrations of protein, lipid (fats), vitamins and minerals (Cochrane and Duffus, 1981; Evers and Millar, 2002). In the barley grain, the embryo and endosperm are surrounded by an epidermis consisting of the testa and the pericarp which are in turn surrounded by a hull consisting of the lemma and palea. In barley these hull structures adhere to the pericarp at maturity (Evers and Millar, 2002). In terms of evolutionary survival a grain is the basin or vessel where the embryo and endosperm develop so perhaps the presence of one or both of these structures, however small, justifies its inclusion as a grain for the purposes of scientific investigations interested in the yield components.

Post-fertilisation development can be considered in three phases: (1) a period of cell division during which most of the cells of the endosperm are formed (Kirby and Appleyard, 1984); (2) a period of rapid grain filling when the grain accumulates dry matter (Slafer et al., 2009); (3) a ripening period of grain dehydration prior to harvest.

Endosperm cell division in barley can continue for up to 30 days post-anthesis (Cochrane and Duffus, 1981, 1983; Evers, 1970; Kvaale and Olsen, 1986; Nicolas et

al., 1985; Radley, 1978) however the division of starchy type endosperm cells, which contribute most to grain weight, ceases approximately 14-23 days after anthesis after which cell division of aleurone cells only continues (Cochrane and Duffus, 1981, 1983; Kvaale and Olsen, 1986; Olsen and Krekling, 1980). Aleurone cells are unlikely to provide further capacity for carbohydrate storage and hence yield. During this phase of development ear growth rate is less than total crop growth rate and as such excess assimilate is likely to be stored elsewhere (Gallagher et al., 1975) e.g. in the stem.

Grain growth begins, approximately 10 days post-anthesis (Gallagher et al., 1976) when large amounts of photosynthate begin to be deposited in the developing reproductive structures (Zinselmeier et al., 1999). Cell growth and differentiation continue as deposition of starch begins, and at the same time the fertilised egg gives rise by cell division to the embryo. (Kirby and Appleyard, 1984). During grain filling, dry matter accumulates rapidly as does grain water content (Slafer et al., 2009). Rapid dry matter accumulation is largely linear (Smith et al., 1999) and often grain growth rate exceeds that of total crop growth rate so additional stored assimilate is utilised alongside current photosynthesis (Gallagher et al., 1975) to meet the demand of the growing grains. As such, assimilate is translocated to the growing grain (via the phloem) from current photosynthate and/or storage reserves, however photosynthesis of the ear itself can contribute a substantial amount to the pool of reserves available for grain filling (Evers and Millar, 2002; Serrago et al., 2013). Starch is synthesized from this pool of soluble reducing sugars (Baxter and Duffus, 1973) of which sucrose is a major component (Duffus and Cochrane, 1992; Felker et al., 1984; Gubatz and Shewry, 2011). Starch comprises 60-70 % of final grain dry weight (Duffus and Cochrane, 1992) and cell size, starch granule size, and starch concentration increase towards the center of the endosperm (Evers and Millar, 2002). It is unclear whether this is due to central cells (first formed) having had longer to produce storage products, or whether it is simply an inherent product of varying cell differentiation dependent on location in the endosperm (Evers and Millar, 2002).

This period of grain dry matter accumulation can last 24-51 days in cool moist UK conditions (Bingham et al., 2007b; Gallagher et al., 1976; Newton et al., 2011).

Physiological maturity is reached at the maximum dry matter accumulation stage and signals the end of grain filling which is then followed by a ripening stage prior to harvest (Slafer et al., 2009).

At this point in the chapter the development of the barley crop has been tracked from germination through to harvest. The circular ontogeny of barley grains is summarised below in Figure 1.2.

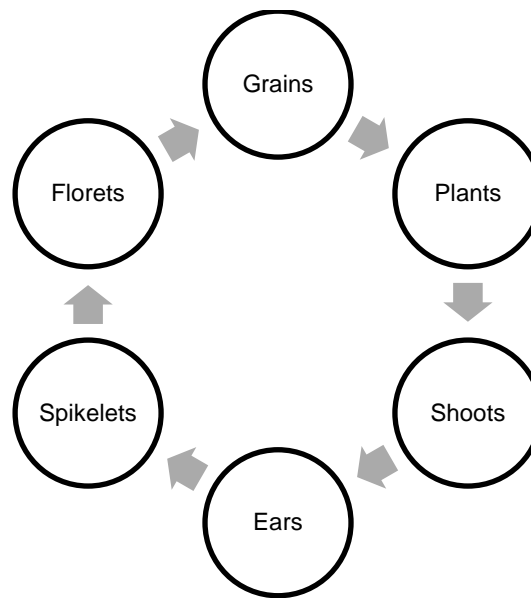


Figure 1.2. Ontogeny of barley grains

1.8 Yield components

From a purely physical viewpoint, yield of barley (*Hordeum distichum* L.), and any grain crop, can be expressed in terms of yield components. Grain yield per unit area is expressed as a product of ear number per unit area, grain number per ear and mean grain weight (MGW) (Gallagher et al., 1975). This equation can be further simplified:

$$\text{Yield} = \text{Grain Number} \times \text{MGW}$$

Grain number in barley grown in a range of environments is highly correlated with yield (Abeledo et al., 2003; Baethgen et al., 1995; Bingham et al., 2007a; Blake et al., 2006; del Moral et al., 2003; Gallagher et al., 1975; Peltonen-Sainio et al., 2007;

Serrago et al., 2013). This is due to the influence of ear number (Abeledo et al., 2003; del Moral et al., 1984; del Moral and del Moral, 1995; Gallagher et al., 1975; Grausgruber et al., 2002; Kren et al., 2014), but also through the influence of grain number per ear (Arisnabarreta and Miralles, 2008b; Baethgen et al., 1995; Gallagher et al., 1975). This has led to the conclusion that grain number largely determines yield in barley. Sections 1.4 and 1.5 indicate that grain number is largely determined prior to anthesis which implies that yield is largely determined prior to anthesis (Smith et al., 1999). However, this may not strictly be the case – mechanisms controlling grain number will be discussed in greater detail in section 1.10 below.

Sinclair and Jamieson (2006) argue that both yield and grain number are constrained by a crops ability to gather resources which technically is true – every organ of a barley plant is source limited at some stage in its development. However the claim by Sinclair and Jamieson (2006) that grain number is more a consequence of yield than a determinant is opposed by Fischer (2008) who argues that the commonly accepted approach that yield components and source-sink balance can determine yield is a valid model for physiological investigations of yield potential.

There is less of a relationship between grain weight and yield – grain weight tends to be quite conserved across a range of yields (Abeledo et al., 2003; Baethgen et al., 1995; Blake et al., 2006; Bulman et al., 1993; Gallagher et al., 1975; Sadras and Slafer, 2012; Wade and Froment, 2003). This is unsurprising given that photosynthate supply is unlikely to be limited post-anthesis (see section 1.1). In eighteen site/seasons of data from winter barley crops in the United Kingdom (UK) managed for high yield potential MGW showed a 32% variation (from maximum to minimum) compared to a 70% variation in grain number (Blake et al., 2006). This conservation of seed size relative to seed number is also a feature of other crops including wheat (Borrás et al., 2004; Fisher, 1975; Slafer and Savin, 1994), maize (Borrás et al., 2004; Borrás et al., 2003) and soybean - analysis by Sadras (2007) of studies on soy bean over 19 years in the USA by Kelley et al. (2003) showed a range in variation of seed size of 43% compared with a range of 291% for seed number. However variation in seed size is increased if resource availability is strongly reduced during grain filling (Borrás et al., 2004); particularly in stressful

environments (Blum, 1998). Grain weight is a trait that is susceptible to genetic improvement but past breeding programs have tended not to affect it (Abeledo et al., 2003) most likely because breeding historically has been largely focused on yield improvement and it appears grain number has the strongest influence on yield. Genetic and agronomic control of grain weight in barley will be discussed in more detail in section 1.11.

1.9 Assimilate supply for grain filling: production, temporary storage and remobilisation

Grashoff and d'Antuono (1997) quantify the weight gain of an organ between two samplings as the sum of the current assimilate produced and accumulated during that period plus translocation of assimilate from other organs. Assimilate for dry matter production during grain filling is derived from both post-anthesis photosynthetic activity and remobilisation of storage reserves deposited pre-anthesis (Bingham et al., 2009; Gallagher et al., 1975; Nicolas et al., 1985). Pre-anthesis storage reserves are mainly in the form of soluble carbohydrate reserves present in the stem at the time of ear emergence (Horrie et al., 1997).

Post anthesis photosynthetic activity includes direct assimilation to the grain but also further deposition of storage reserves early in the period when production exceeds grain demand (Gallagher et al., 1975). Bingham et al. (2007a) quantify the potential assimilate supply for grain filling as:

$$_{int}PAR \times RUE + \text{soluble carbohydrate storage reserves}$$

Where $_{int}PAR$ is the amount of photosynthetically active radiation intercepted by green tissue post-anthesis and RUE is the radiation use efficiency of the crop. Carbohydrate demand from the growing grains can influence the level of utilisation of assimilate available (Grashoff and d'Antuono, 1997; Radley, 1978) and RUE and photosynthetic efficiency post-anthesis may be regulated by the number and storage capacity of the grains (sink size) in wheat and barley (Bingham et al., 2007a; Calderini et al., 1997; Miralles and Slafer, 2006; Reynolds et al., 2005). PAR_{int} ,

which allows for green area decline post-anthesis, factors in any possible hastening of senescence due to a restricted sink capacity.

Senescence in plants is a terminal biological process leading to the ultimate attrition of a leaf or any plant part and the first visible sign is the breakdown of chlorophyll (Gan and Hörtensteiner, 2013). Senescence is a phase of plant development involving the degradation of photosynthesising tissues and subsequent remobilisation of reserves, particularly nitrogen, to the developing grain (Gregersen et al., 2013; Gregersen et al., 2008; Humbeck et al., 1996; Parrott et al., 2005). It can begin as early as eight days post-anthesis in field-grown barley (Humbeck et al., 1996). In monocarpic plants (those that reproduce once and then die e.g. cereals) reproductive structures often govern senescence of the whole plant (Nooden et al., 1997). It has been demonstrated that high carbohydrate levels (sugar accumulation) in leaves are associated with the onset of senescence in barley (Parrott et al., 2005) and other species (Masclaux et al., 2000; Nooden et al., 1997; Yoshida, 2003). In such a situation, sugars may act as signaling molecules (Rolland et al., 2002) initiating or accelerating the breakdown of Rubisco and programmed cell death associated with senescence. It is therefore possible that a restricted sink demand from developing grains can hasten senescence through a feedback inhibition of post-anthesis photosynthesis. Premature senescence is also induced by stress such as drought, low nitrogen supply, high temperature, biotic stress and high/low light intensities (Gregersen et al., 2013; Jamieson et al., 1995; Parrott et al., 2005). However senescence is also strongly under genetic control (Fangmeier et al., 2000; Nooden et al., 1997; Smart, 1994) and as such has potential for manipulation to enhance productivity in environments where delayed senescence or ‘stay-green’ traits may be particularly useful (Wu et al., 2012). However, if sink strength is the major limiting factor for yield then breeding for enhanced green area duration to increase post-anthesis assimilate supply should be accompanied by larger or stronger sink (Gregersen et al., 2013) to avoid inefficient photoassimilate remobilisation and a lower harvest index (Gong et al., 2005).

(Biscoe et al., 1975b) identified the flag leaf sheath and the ear as the largest contributors to canopy photosynthesis during the approximate rapid grain filling

period of UK grown spring barley (cv. Proctor). The flag leaf sheath was the largest, contributing 35 % of net photosynthesis during the period which can be attributed in some part to its prolonged green area (Biscoe et al., 1975b). Serrago et al. (2013) have shown the potential contribution of direct ear photosynthesis to grain filling where post-anthesis shading was imposed to leaves but not to ears of barley and wheat in a Mediterranean environment – reductions in grain weight were non-significant or minor and spike photosynthetic rate increased to almost fully compensate for the shading of the rest of the canopy. However, Biscoe et al. (1975b) estimates that only 70% of final grain weight in barley is accounted for by photosynthesis of the canopy after anthesis.

Stem soluble carbohydrate storage reserves are deposited mostly as hexoses and fructans (Archbold, 1942; Thomas, 1977) both pre- and early post-anthesis. These can later be remobilized and utilised for grain filling in barley and other crops (Beed et al., 2007; Fabian et al., 2011; Foulkes et al., 2007; Serrago et al., 2013; Yoshida, 1972). The contribution of storage reserves to grain filling can vary with environment, season and sink size (Gallagher et al., 1975). Stem reserves may only be utilised if the photosynthetic capacity of the crop is reduced post-anthesis (Grashoff and d'Antuono, 1997; Nosberger and Thorne, 1965; Serrago et al., 2013) or the crop is subject to heat or drought stress (Bell and Incoll, 1990; Blum et al., 1994; Davidson and Chevalier, 1992; Ehdaie et al., 2006). In such cases, stored carbohydrate reserves may act as a buffer to maintain a steady rate of grain filling (Ehdaie et al., 2006). Grashoff and d'Antuono (1997) demonstrate the sensitivity of stem reserves to current growth conditions in spring barley as relatively short periods of shading caused a large reduction in soluble carbohydrate reserve concentration in the leaves and stems. Also, concentration will vary depending on what time of day samples are taken and the level of respiration at that time.

When assimilate supply for grain filling was estimated as per Bingham et al. (2007a) for winter barley crops across eighteen site/season combinations in the UK, all but one had a potential supply exceeding grain yield suggesting that crops were predominantly sink limited. It appears that current assimilate from photosynthesising green area is the preferred source of carbohydrate for grain growth and only when

this is restricted are stem reserves drawn upon. The strong buffering availability of storage reserves for dry matter accumulation during grain filling support the suggestion that there is an excess of post-anthesis assimilate availability in barley.

1.10 Control of grain number

Grain number per unit area largely determines yield per unit area and has two sub-components – ear number per unit area and grain number per ear. Ear number per unit area is consequent of the number of fertile tillers and main stems per unit area that reach anthesis and survive into grain filling and maturity. Likewise, grain number per ear is consequent of the number of fertile spikelets per ear that are fertilised at anthesis and proceed to accumulate dry matter to become mature grains at harvest. Tiller initiation normally, but not always, completes at the same stage as spikelet initiation completes – pre stem-extension when the embryo ear is still at ground level enclosed by developing leaves. At this early stage in the plants lifecycle the maximum potential size of two of the grain number sub-components are already determined. The relative importance of each sub-component of grain number will be discussed along with mechanisms, periods and influencers determining their final harvest values. Building on previous sections, the plasticity and possible overlap of determination periods and trade-off between these two sub-components will also be discussed.

Duration of tillering is influenced by environmental factors discussed in section 1.2. Tiller production was negatively associated with mean air temperature during tillering in a Mediterranean environment indicating that low temperature favours tiller production by slowing the growth of leaves, tillers, and spikes thereby reducing competition for a limited supply of resources (del Moral and del Moral, 1995). High rates of nitrogen fertiliser applied early in the season (mid-tillering) can stimulate tillering (Baethgen et al., 1995; del Moral et al., 1984) but excessive tillers may fail to produce ears Baethgen et al. (1995). During stem extension there is competition between plant structures for a limited pool of resources (see sections 1.4 and 1.5). As such survival of sink components may become more important than initiation – in the

study by (Baethgen et al., 1995) nitrogen fertiliser applied around stem extension had the greatest influence on grain number at harvest. Nitrogen availability during stem-extension was related to shoot mortality in a nitrogen timing and rate field experiment on barley in Brazil (Wamser and Mundstock, 2007). Sylvester-Bradley et al. (2001) have shown that large N residues in wheat had a greater effect on shoot survival than shoot production and almost always led to more shoots at harvest. A reworking of data from Widdowson et al. (1987) by Sylvester-Bradley et al. (2001) has shown that in winter-sown wheat the average nitrogen content per live shoot might be relatively stable at 2 mg. Sylvester-Bradley et al. (2001) propose that for a target shoot production number of 1200 shoots m⁻² crops would as such need a N content of at least 24 kg ha⁻¹ N before the end of tillering to ensure adequate shoot survival. There is a close relationship between soil nitrogen supply in the spring and shoot survival in UK grown winter-sown wheat (Sylvester-Bradley et al., 2001). Water availability also has the potential to influence tiller dynamics. Drought can result in a reduction in radiation use efficiency and hence biomass production (Jamieson et al., 1995) and as such can reduce the pool of resource available at any particular time potentially resulting in tiller mortality. Drought treatments imposed during stem-extension in spring barley pot experiments resulted in a reduction in tiller number (Svobodova and Misa, 2004). These reductions were greater than a similar treatment imposed pre-stem-extension where a flush of mid-late season tillering followed the removal of the pre-stem-extension drought treatment (Jamieson et al., 1995). However, plants that tiller late produce fewer and smaller tillers with less chance of survival (Thorne and Wood, 1988).

While light intensity via its strong influence on assimilate supply will have a bearing on tiller production and mortality, several authors argue that light quality, particularly light quality lower in the canopy, is of greater importance (Davis and Simmons, 1994; Lauer and Simmons, 1989; Sparkes et al., 2006). A lowered red to far-red light ratio associated with reflection of far-red light from neighbouring plants has been shown to both reduce tiller production (Davis and Simmons, 1994; Skinner and Simmons, 1993) and promote tiller mortality (Sparkes et al., 2006) and this may be mediated by leaf nitrogen concentration (Sparkes et al., 2006; Zhong et al., 2002). Tiller senescence has been shown to begin both pre- and post-stem-extension in

barley and wheat (Lauer and Simmons, 1989; Sparkes et al., 2006) and there may be a critical leaf area index and red to far-red ratio at which this occurs (Sparkes et al., 2006; Zhong et al., 2002).

Plant density also has an influence on tiller dynamics whereby tillering begins earlier and happens at a higher rate at lower plant densities (Kirby and Faris, 1972). Also, early-season (pre-stem extension) applications of plant growth regulators (hormone based agrochemicals) in spring barley can suppress the development of main stem spikes by imposing a delay on main stem apical development (Ma and Smith, 1991). This can temporarily lessen the dominance of the main stem spike and promote enhanced growth and survival of later produced tillers (Smith et al., 1999) thus potentially increasing final ear number at harvest. Varietal differences also exist in terms of tiller production as well as survival rates (Cannell, 1969; Kirby, 1967; Thorne, 1962). Matching the tillering potential of the cultivar to the environment in question is important to maximize both ear number and resource use efficiency.

After the formation of the ear (as discussed in section 1.4) is complete, some of the spikelets or florets initiated will die at a young age during stem elongation (Kirby, 1977; Kirby and Appleyard, 1984; Waddington and Cartwright, 1983) and prior to anthesis (Cottrell et al., 1985; Gallagher et al., 1975; Kirby, 1973). This spikelet death period runs concurrent to the period during which tiller death can occur. The rapid ear growth period is crucial for grain number determination in wheat also (Abbate et al., 1997; Fischer, 1985; Miralles and Slafer, 2007; Reynolds et al., 2000). This occurs during stem extension where significant spikelet mortality can occur due to a shortage of photosynthate (Arisnabarreta and Miralles, 2008a; Richards, 2000) thus reducing grain number per ear (Kirby and Jones, 1977). Overall grain number may be increased by increasing spikelet growth during the stem-extension period by lengthening its duration (Abeledo et al., 2003; Miralles et al., 2000; Miralles and Slafer, 2007; Reynolds et al., 2009). Slower floret development results in more potential grain sites (Miralles and Slafer, 2007). Lengthening the stem elongation period would need to be achieved without producing major changes in the total period from sowing to anthesis to avoid a constricted grain filling period and/or late harvest (Reynolds et al., 2009) – manipulation of genetic sensitivity to photoperiod is

one possible approach (Slafer et al., 2001). Alternatively, increasing pre-anthesis RUE, thereby making more assimilate available to increase spike mass, may avoid unnecessary floret death during the stem extension period (Reynolds et al., 2009). Aside from assimilate availability, a pre-emptive signaling strategy may be responsible for the pre-anthesis death of potential florets in wheat in response to environmental conditions e.g. variation in levels of light interception (Fischer, 1985; Reynolds et al., 2009). Floret survival in wheat can be particularly sensitive to environmental conditions, particularly light interception and particularly during the relatively narrow developmental window of rapid spike growth (Abbate et al., 1997; Fischer, 1985).

Source:sink manipulation experiments are useful in determining the relative importance and contribution of certain developmental periods to yield formation and yield component determination. Shading in the field or controlled environment can manipulate radiation levels and temporarily reduce the supply of photosynthetic assimilates available to the crop or plant in a controlled manner and for a fixed period of time (Estrada-Campuzano et al., 2008; Fisher, 1975; Grashoff and dAntuono, 1997; Jenner, 1980; Willey and Holliday, 1971). Thinning treatments whereby some sinks and/or sources are physically removed from the crop or plant can artificially modify the amount of assimilate available to the remaining sinks by a fixed amount (Habgood and Uddin, 1983). These mutilation treatments are generally permanent modifications in the source-sink balance. Assessing resultant growth can infer details on the source:sink balance of the crop and the relative importance of the treatment period for yield and yield component determination.

Shading reduced radiation by 60% to spring barley in the field across two growing seasons in the Netherlands during tillering, during stem-extension, and from ear emergence to maturity (Grashoff and dAntuono, 1997). These treatments reduced yield by 5%, 35% and 45% respectively. Whilst shading during tillering did reduce the number of tillers per unit area during the treatment period, tiller number recovered once the shades were removed and thus had no effect on final grain number per unit area. Shading during stem elongation reduced final grain number by about 40% due mainly to a 35% reduction in the number of grains per ear. Shading

from ear emergence to maturity reduced grain number by about 19% mainly through a 16% reduction in ear number however the large yield reduction was mainly due to a reduction in MGW. UK shading studies on spring barley by Willey and Holliday (1971) affording 28% and 54% reductions in solar radiation during three developmental phases similar to Grashoff and d'Antuono (1997) show a slightly different pattern of yield reduction. The early shading treatment (during tillering) produced moderate yield reductions mainly as a consequence of decreased tiller production. The intermediate shading period (during stem elongation) produced the largest yield reductions, through reductions in all yield components but in particular through reductions in grain number per ear. Interestingly, the later shading treatment had no significant effect on grain yield – despite a small and significant decrease in grain weight this was compensated for by a slight increase in ear number. This lack of yield effect of the later treatment is in contrast to Grashoff and d'Antuono (1997) and indicates that the study crop of Willey and Holliday (1971) was heavily sink-limited. These results show that grain number can be impacted upon over a long period of development and that certain compensatory capacity as regards grain number determination exists within the crop.

Experiments on triticale (*X Triticosecale* – a hybrid of wheat and rye) in South America involving five shading treatments applied at various periods from the beginning of tillering until physiological maturity showed that the largest yield reductions came from shading applied two weeks pre-anthesis to one week post-anthesis (Estrada-Campuzano et al., 2008). Shading reduced incident light by 67 % and yield reductions from treatments pre-anthesis were fully explained by changes in grain number and most consistently by grain number per ear rather than ear number. In the post-anthesis treatment there was a greater effect on grain number than grain weight. This indicates that grain number can be impacted upon right up to and even beyond anthesis. Also the relatively small effect of post-anthesis shading on grain weight (a 7-14% reduction in grain weight in comparison to a 67% reduction in radiation) would also point towards a sink-limitation of grain filling in triticale. As with Willey and Holliday (1971), the importance of grain number and in particular grain number per ear is highlighted. The strong relationship found between the final number of grains per m² and the photothermal quotient for the period of thirty days

prior to anthesis gives some indication of the critical period for grain number determination, at least in triticale. Crop thinning by removal of alternating crop rows was carried out by Habgood and Uddin (1983) on UK barley crops with thinning effects applied over two periods: from maximum floret primordia stage for 8-10 days (during early stem-extension), and from anthesis to physiological maturity. While the percentage increase in radiation intercepted was not quantified both treatments increased the number of grains per ear and overall yield per shoot, with the earlier treatment having the greatest effect. Habgood and Uddin (1983) and Estrada-Campuzano et al. (2008) have also shown that it is possible to impact upon grain number post-anthesis.

Other source: sink manipulation experiments on barley and other small grain crops have shown the potential to influence grain number post-anthesis (Beed et al., 2007; Grashoff and d'Antuono, 1997; Nicolas et al., 1985; Zinselmeier et al., 1999) mainly via a reduction in the number of grains per ear. Post-anthesis grain number effects may be due to interference with pollination and fertilisation early post-anthesis – when wheat plants were drought treated post-anthesis in a controlled environment grain sterility was higher than the control (Fabian et al., 2011; Nicolas et al., 1985). Such reduced grain set tends to be in the distal part of the ear only, because distal spikelets flower 2-3 days later than spikelets in the central portion of the ear and thus were in the early stages of cell division when the drought became severe (Nicolas et al., 1985). Also Serrago et al. (2013) has shown that grain number was not modified significantly by shading of the leaves or whole canopy from 7 days after anthesis (the period during which you would expect pollination and fertilisation to be complete). However it is also possible that a grain abortion mechanism acting after pollination is responsible for post-anthesis grain number adjustment – water deficits post-pollination in maize can inhibit photosynthesis and trigger abortion of ovaries through the depletion of ovary sugar pools (Boyer and McLaughlin, 2007; Boyer and Westgate, 2004; Zinselmeier et al., 1999). Several genes are responsible for this (Boyer and McLaughlin, 2007) but many developing embryos can be rescued by feeding sucrose to the stem of affected plants (Boyer and Westgate, 2004; Zinselmeier et al., 1999). Similarly to senescence (as discussed in section 1.9), sugar signaling (Rolland et al., 2002) may be responsible for this potential down-regulation

of grain number post-anthesis. While grain number is mostly regulated pre-anthesis and at anthesis, adjustments to the eventual number of grain sites available for grain filling may occur post anthesis.

Arisnabarreta and Miralles (2008b) conclude that the number of grains per ear has a strong bearing on overall grain number. However this may be less so in two-row type barleys where the influence of the number of ears per unit area will have a stronger bearing on overall grain number as two-row barleys are limited by the number of grains per ear they can bear (Arisnabarreta and Miralles, 2008a). Two-row barley types tend to tiller more than six-row varieties due mainly to early developmental differences (Kirby and Riggs, 1978) whereby a larger main shoot apex in six-row varieties may increase the dominance of the main shoot over later formed tillers (Kirby and Jones, 1977). Two-row type barleys tend to produce more leaves on the main stem than six-row types and because tiller buds emerge from the axils of leaves they will have a greater tillering potential (Kirby and Riggs, 1978). Two-row types produce more ears at harvest than six-row (del Moral and del Moral, 1995) and six-row barley types have more grains per ear than two-row types but a lower grain weight and, as stated, less ears per plant (Riggs and Kirby, 1978). Which barley type achieves the highest yield will depend upon environmental conditions (del Moral et al., 2003; del Moral and del Moral, 1995). The yield of two-row type barley is more responsive to environmental changes than that of six-row type due to its increased tillering capacity (del Moral et al., 2003; del Moral and del Moral, 1995). Attempts by breeders to combine the best characteristics of both phenotypes have been largely unsuccessful with crosses resulting in low numbers of grains per ear and unacceptably small grains (Riggs and Kirby, 1978).

There is a degree of overlap between the determination periods of ear number and grain number per ear. The same factors controlling grain number per ear may be responsible for the control of ear number per unit area. Also, the overlap of ear number and grain number per ear determination periods means that there is a certain degree of trade-off between the two sub-components. When nitrogen application to spring-sown barley in Uruguay was delayed until just before stem extension the number of grains per ear increased but this was in part due to a reduced tiller number

(Baethgen et al., 1995). Field experiments on barley have shown that at higher plant populations ear number per unit area will increase but above a given plant population attempts to increase overall grain number through increasing ear number will be counteracted by a decreased grain number per ear (Wade and Froment, 2003; Willey and Holliday, 1971). It has been suggested that grain number per ear is more conserved in barley than in wheat, in that wheat can compensate for low tiller numbers by increasing grain number per ear due to the high number of potentially fertile florets per spikelet in wheat (Wade and Froment, 2003). Barley has potential to do this, but not to the same extent as wheat because each spikelet contains only one floret as opposed to several in wheat (Wade and Froment, 2003).

The importance of grain number in producing a high yielding barley crop has been highlighted as has the importance of the pre-stem extension period for tiller production. The stem-extension period for tiller number and grain number per ear survival appears to be equally important. Also, it is clear that the tillering period is quite plastic (Simmons et al., 1982; Willey and Holliday, 1971), something which has a strong bearing on the number of ears m^{-2} at harvest.

1.11 Control of grain weight

As discussed in section 1.7, grain development and grain filling occur post-anthesis implying that mean grain weight at harvest (MGW) is determined during this period (Ellis and Kirby, 1980; Grashoff and d'Antuono, 1997; Scott et al., 1983). However as discussed in section 1.9, MGW may not be simply a function of the amount of photosynthate produced during the post-anthesis period. Grain filling involves the utilisation of stored carbohydrate reserves and there may be a relationship between assimilate production and sink demand. MGW control can be considered not only in terms of source but also in terms of sink. The grain development phase occurring early post-anthesis and overlapping with the grain filling phase may impact upon the sink demand of the grains through its influence on grain storage capacity. Also, grain number per unit area, the determination period of which is quite plastic (section 1.10), affects the source-sink balance of the crop. These two sink components (grain

storage capacity and grain number) may determine assimilate supply for grain filling through some sort of feedback mechanism (Nicolas et al., 1985) down regulating RUE and PAR_{int} post-anthesis (section 1.9). These factors will be discussed below with a focus on the control of MGW.

It has already been established that grain weight is the most conservative of the two yield components (section 1.8) and Gallagher et al. (1975) identifies two possible reasons for this: (1) Crops are able to regulate dry matter supply to the grains from a vast pool of assimilate to reach a nearly constant MGW irrespective of grain set; (2) Crops can abort grains post-anthesis to ensure that the remainder are adequately filled and no small grains are produced. Relatively consistent MGW's may be a result of a consistently sink limited crop as per possibilities (1) and (2) or there could be a third reason: (3) Small under-filled grains are consistently lost from harvest grain samples post threshing and separation of chaff. A combine harvester uses a series of adjustable threshing mechanisms, sieves and fans to get a 'clean' sample of marketable and usable grains. In doing so, smaller and lighter grains similar in physical properties (especially weight) to that of the chaff, may be lost from the harvested sample. Such a systematic inaccuracy could result in bias (overestimation) when estimating MGW (Bloom, 1985; Gallagher et al., 1975). For example, if assimilate for grain filling becomes limiting in higher grain number crops resulting in small under-filled grains at harvest, these grains may be lost from the sample resulting in a skewed grain weight distribution, and as such contributing to a relatively conserved MGW. While possibility (3) may explain the lack of grains with weights in the lower end of the grain weight distribution, it does not explain the restricted spread at the upper end. A further possible explanation (4) is that rather than grain number determining yield, yield may simply be a consequence of resource accumulation and use by the crop and grain number may be adjusted to match the resource defined yield level (Sinclair and Jamieson, 2006). If grain number is largely determined pre-anthesis then this would require some means of 'predicting' potential post-anthesis assimilate supply before flowering. This might involve resource-based mechanisms in which floret and tiller survival are regulated by assimilate availability and/or crop growth rate during late stem extension (Sadras and Slafer, 2012).

Gallagher et al. (1975) dismiss possibility (2) because the evidence at the time suggested that grain number per unit area was firmly fixed at or shortly before anthesis. However more recent evidence has shown that the grain number determination period is quite plastic and that a post-anthesis grain number adjustment mechanism may exist in distal positions of the ear or on tillers of less favourable hierarchy (see section 1.10). As such possibility (2) is a plausible explanation for a relatively conserved MGW. If possibilities (1) and/or (2) are responsible for the relatively conserved MGW that exists, then this implies a sink limitation to grain filling accompanied by an excess of assimilate. As seen in section 1.9 stem carbohydrate reserves are available for grain filling. The ability of barley to translocate stem reserves to the grain is one of the reasons for MGW being more stable than other components (Hadjichristodoulou, 1990). These stem reserves may simply remain underutilised if no deficit exists (Gallagher et al., 1975). Gallagher et al. (1975) argue that the potential influence of translocation of storage reserves in conserving MGW is so large that it outweighs the influence that a post-anthesis grain number adjustment might have. Either way an excess of assimilate for grain filling is implied. This excess will undoubtedly differ depending on the site, season and crop yet MGW remains relatively conserved compared to the variation in grain number. In this instance grain weight could be controlled by a restricted grain storage capacity set prior to grain filling rather than the carbohydrate supply throughout (Bingham et al., 2007a; Sadras, 2007; Willey and Holliday, 1971).

Positive correlations have been shown between the number of endosperm cells and MGW in wheat (Brocklehurst, 1977; Gleadow et al., 1982; Hasan et al., 2011; Nicolas et al., 1985) and barley (Cochrane and Duffus, 1983). In UK grown barley, Bingham et al. (2007b) found a significant positive linear relationship between MGW and the amount of photosynthetically active radiation (PAR) intercepted per unit grain number between ear emergence and the start of rapid grain filling – the period during which you would expect the basis of grain storage capacity to be set. Asana and Williams (1965) have shown in an experiment on two Australian wheat cultivars, that the cultivar with the higher MGW also had a greater capacity for growth from the earliest stage. Thinning treatments applied at anthesis to crops of UK grown spring-sown barley produced greater increases in MGW (+ 5 mg) than

thinning treatments applied at 20 days post-anthesis (+2.3 mg) (Habgood and Uddin, 1983). Stimulation of endosperm cell division early in grain development could increase the capacity of the grain to accumulate dry matter and as such MGW (Cochrane and Duffus, 1981).

In wheat pot trials, variations in MGW between spikelets was related to the number of endosperm cells formed (Nicolas et al., 1985). Grain dry weight at maturity was 33% lower in plants drought treated for a period of twenty days post-anthesis (i.e. the approximate cell division period) and there was no increase in growth rate following re-watering (Nicolas et al., 1985). At twenty days post-anthesis only 35% - 45% of final grain dry weight had been accumulated but at this point the sink size of the grains was determined to a large extent as the number of endosperm cells were fixed (Nicolas et al., 1985). In other experimental work on two winter wheat varieties, Evers (1970), found that the increase in the size of the endosperm after sixteen days involves only cell enlargement and not cell division. However Wallwork et al. (1998) has shown that in barley, following a five day period of elevated temperature treatment from sixteen days after anthesis, cell division continued and at a rate faster than the synthesis of endosperm starch.

Wheat plants subjected to 5-10 day periods of shading in a controlled environment immediately post-anthesis resulted in lower rates of dry matter accumulation in the grains and a lower MGW Jenner (1979). Shading for the 5 days immediately post-anthesis had no statistically significant effect on MGW or grain size while shading from 5 days post-anthesis until 10 days post-anthesis did (Jenner, 1979). While the crucial period for starchy endosperm cell number determination is early during grain development there may be a slight time-lag before it begins in earnest. Following removal of some spikelets from the shaded ears after the shading treatment i.e. artificially reducing the sink size to increase the assimilate available per grain, MGW of the remaining spikelets did not increase (Jenner, 1979). This indicates that the MGW reduction due to shading was not simply a consequence of depleted levels of assimilate per grain for grain filling and likely a consequence of a reduced endosperm cell number or grain storage capacity (Jenner, 1979). Fabian et al. (2011) have also shown that drought stress from the fifth to the ninth day after pollination in

two winter wheat cultivars affected the subsequent rate of grain filling, shortened the duration of grain filling and severely reduced yield. Yield was reduced through both a reduction in grain set and in MGW where the number of starch granules in the endosperm and mature embryo size were significantly reduced (Fabian et al., 2011). Effects on MGW in this instance were attributable to differences in endosperm cell number but also to a reduction in cell growth from a reduced source – the transport of assimilates was interrupted following drought damage to vegetative tissues.

The flow of assimilates to the grain and associated mechanisms have a role to play in the control of MGW. There are two types of transport tissue in vascular plants the xylem which is involved in the upward transfer of water and the phloem which transports nutrients and sugars. Sucrose supports cell development and synthesis of starch. However, Jenner (1980) demonstrates in wheat that the inflow of sucrose into the grain is not dependent on amount of sucrose supply - trimming ears to leave four remaining spikelets resulted in an increase of sucrose in the rachis and peduncle i.e. the supply to the grain, but an actual depression of sucrose levels in the endosperm. When detached ears of wheat were cultured on solutions of sucrose, starch synthesis continued in the endosperm but despite the large availability of potential assimilate the concentration of sucrose remained constant in the endosperm while it reached higher levels in other organs of the ear (Jenner, 1970; Jenner, 1973; Jenner and Rathjen, 1972a). This led to the conclusion that the level of sucrose in the grain is not limited by the production of assimilate but by a limitation imposed by the sucrose transport mechanism on the final stages of its passage into the endosperm (Jenner, 1970; Jenner, 1973; Jenner and Rathjen, 1972a) so long as the supply of sucrose into the grain is at its upper limit (Jenner and Rathjen, 1972b).

There is evidence in wheat and barley that MGW may also be influenced by events shortly before anthesis. MGW has been found to be positively related to carpel weight at anthesis (Calderini et al., 1999; Calderini and Reynolds, 2000; Hasan et al., 2011; Scott et al., 1983) and is positively influenced by cool temperatures during the booting stage immediately pre-anthesis (Bingham et al., 2007b; Calderini et al., 1999; Calderini et al., 2001) perhaps via an increase in the duration of carpel development (Bingham et al., 2007b; Calderini et al., 2001). It is possible that hull

size and weight are limiting factors to grain growth (Habgood and Uddin, 1983; Scott et al., 1983). Trichomes, or hairs, covering the entire surface of the carpel prior to fertilisation are visible at the distal end of the mature barley grain as a brush like structure providing evidence of the link between seed coats and carpel characteristics (Evers and Millar, 2002). Grain weight can possibly be impacted upon as early as the tillering stage – in field trials on UK spring-sown barley where certain tillers were removed as they emerged MGW was increased on remaining tillers possibly due to an increased ovary size due to the reduced competition for resources (Kirby, 1977; Kirby and Jones, 1977).

While MGW across sites and seasons is certainly conserved, grain number does not account for all of the variation in yield – there is some degree of variation in MGW. MGW decreases with increased tiller hierarchy (Evers and Millar, 2002; Gallagher et al., 1976; Habgood and Uddin, 1983; Naylor et al., 1998) and distance from the centre of the spike (Evers and Millar, 2002). MGW is greatest for the middle grains of an ear and tapers gradually towards the basal and distal ends (Scott et al., 1983). This is due to differences in growth rate and duration depending on the location on the ear (Kosemarno and Sedcole, 1994; Smith et al., 1999). This particular variation in MGW is not easily explained in terms of competition between grains for assimilates during grain filling (Bremner and Rawson, 1978; Miralles and Slafer, 1995; Voltas et al., 1998). This implies that an inherent difference set earlier during crop development perhaps due to a restricted or delayed development (see sections 1.4 and 1.5) is responsible for the hierarchy of MGW with position within and between ears (Bingham et al., 2007b). Drought for a period of twenty days post-anthesis mostly affected the final dry weight of distal grains of top spikelets in the wheat ear, partly because these grains reached anthesis two-three days later than other grains on the spike and thus experienced the drought period earlier in their development (Nicolas et al., 1985). MGW is less stable in 6-row type barleys (Hadjichristodoulou, 1990) and genotypic variation exists for both rate and duration of grain-filling (Smith et al., 1999) adding to the argument that the variation is not a consequence of a limited supply of assimilates.

Other sources of variation in MGW include seasonal variation (Gallagher et al., 1975) and the growing environment of the crop (Evers and Millar, 2002). In growth analysis data from 17 site/year combinations of UK grown winter barley MGW varied from 35 to 46 mg at 100% dry matter (Bingham et al., 2007b) and variation was associated with rate rather than duration of grain filling. Voltas et al. (1997) have shown that in the low-yielding rain-fed environment of northeast Spain, MGW was positively correlated with yield at eleven sites across four years. But the Voltas et al. (1997) experiments were under varying degrees of water and temperature stress and showed a significant genotype x environment interaction of yield. High-yielding six-rowed cultivars were used that are perhaps less well adapted than stable less productive landraces to the types of stresses mentioned (Voltas et al., 1997). In Ireland growers enjoy relatively cool and long days with little water and heat stress. Significant negative associations of MGW with shoot number per plant, and with mean air temperature from stem extension until the ear was completely emerged suggest that both pre- and early post-anthesis conditions operate in concert to determine the potential grain weight of barley in temperate climates (Bingham et al., 2007b). This implies a possible overlap with the grain number determination period.

There may be a degree of trade-off between grain number and MGW prior to rapid grain-filling (Sadras, 2007). Final MGW was inversely related to grain number in a field experiment in the Netherlands carried out on spring barley where a range of shading and nitrogen treatments were applied at different periods from tillering through to physiological maturity (Grashoff and d'Antuono, 1997). Voltas et al. (1997) illustrate how the source:sink relationship can impact upon grain weight in Northeast Spain where a 50% sink reduction was carried out at anthesis on six-row barley cultivars in the field by piercing half of the florets on a spike with a sharp needle (Voltas et al., 1997). When weights of remaining grains on these spikes were compared to those of control spikes there was an average increase in grain size of 20% (Voltas et al., 1997). Also, Jenkyn et al. (1992) found that in field experiments on winter-sown barley in the UK, increases in grain number per unit area achieved by increasing the seed rate from the 'standard' 300 seeds m⁻² to 450 seeds m⁻² were not matched by yield increases due to a reduction in MGW. However, further UK trial work on spring-sown barley from six site/seasons, four varieties, and five seed

rates, has shown that at very high plant populations, there was not necessarily a decrease in MGW compared to that achieved at optimal plant populations (Wade and Froment, 2003). Habgood and Uddin (1983) provide further evidence that increasing grain number per unit area in the field in UK conditions did not negatively affect MGW adding to the argument that grain yield is largely sink limited.

1.12 Knowledge gaps

Yields of barley in Ireland are high (Anon., 2011a) but variable (Anon., 2012a). It is not known whether the yield potential is close to the theoretical maximum for this environment or whether there may be scope for further improvement through breeding or improved crop management. Also, there is little evidence as to what is driving the high (and variable) yields in the temperate maritime climate of Ireland. Understanding the response of a crop to its environment is an important starting point for physiological investigations on yield and will help springboard more detailed investigations into the mechanisms and processes surrounding yield component determination and the source:sink balance of crops.

There is evidence that yield variation amongst barley crops in a range of environments is related mostly to variation in the number of grains produced (Abeledo et al., 2003; Bingham et al., 2007a; Blake et al., 2006; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013). This has given rise to the view that yield of barley is largely sink limited. It is unclear whether this applies in the high yield potential Irish context. Further grain number increases may be effective in increasing yield potential if grain growth is mainly sink limited but ineffective if the grain number increases were accompanied by grain weight reductions due to a source limitation to grain growth (Foulkes et al., 2011; Reynolds et al., 2009; Serrago et al., 2013; Slafer et al., 2005). If increasing sink capacity is adopted as a method for increasing yield potential then it is necessary to understand what controls sink capacity. Grain sink capacity is a product of the number of grains and their capacity for storing dry matter (grain storage capacity) (Evans and Wardlaw, 1996).

Sadras (2007) and Reynolds et al. (2009) discuss the conflicting needs of plants to produce enough seed to survive on the one hand versus the need to ensure viability of the resultant seed on the other. There may as such be a degree of trade-off between yield components. Sadras (2007) proposes that a highly plastic grain number allows for any potential variability of resources and is responsible for the narrow range of grain weight observed in barley and other crops. This may be the result of evolutionary and/or agronomic selection whereby grain number is down regulated to ensure consistently well-filled grains. If this conservative tendency exists and has become genetically fixed it may represent a bottleneck for achieving genetic gains in optimal environments (Reynolds et al., 2009). Currently our understanding of the relationships between assimilate available for grain filling, grain number, and grain storage capacity is weak which limits our ability to predict the theoretical yield potential.

Grain number is considered to be determined pre-anthesis, but the possible existence of mechanisms to down-regulate grain number after anthesis when assimilate supply is limited needs to be investigated. The presence of a post-anthesis grain abortion mechanism might explain the relatively conserved nature of grain weight in barley and provide an obstacle to further increasing sink size. Both ear number per unit area, and grain number per ear, impact upon total grain number. Their respective influence may depend on the growing environment of the crop and there may also be a degree of trade-off between these two sub-components. The relative importance of either sub-component for determination of overall grain number per unit area is unclear as are the environmental factors controlling tiller and spikelet survival (Davis and Simmons, 1994). The influence of ear number diminishes with higher seed rates due to reductions in the number of grains per ear (Wade and Froment, 2003; Willey and Holliday, 1971) but ear number is readily influenced by agronomic practices (Conry, 1995, 1998; Jenkyn et al., 1992; Wade and Froment, 2003). Where ear numbers are already high there may be greater scope to increase overall grain number by focusing efforts on increasing grain number per ear. The relationships between ear number, grain number per ear, overall grain number and assimilate supply throughout the season need to be better understood. Survival of tillers and spikelets may be of greater importance than initiating more tillers and spikelets.

Initiation is largely completed pre-stem-extension and survival is largely dictated post-stem-extension and pre-anthesis. Previous studies on wheat have identified the stem-extension period as of greatest importance for grain number determination (Abbate et al., 1997; Fischer, 1985; Miralles and Slafer, 2007; Reynolds et al., 2000) due to its influence on floret survival but it is unclear if this applies to barley.

The approximate 16-23 day period immediately post-anthesis is important for determination of endosperm cell number and hence grain storage capacity. However it is also possible that carpel size determined pre-anthesis can influence grain storage capacity and ultimately MGW at harvest. Many of the experiments looking at the determinants of MGW and assimilate availability for grain growth were carried out on pot plants in controlled environments perhaps due to the precision and control needed to look at such finite factors. However it is difficult to predict the behavior of crops in the field from the results of experiments carried out in controlled environments due to differences in light intensities and the interaction between plants at the crop scale (Biscoe et al., 1975a; Gallagher et al., 1983). Field environments can be extremely variable in terms of time and space (Biscoe et al., 1975a). The strength of solar radiation can change by an order of magnitude in just a few seconds as a cloud passes over the sun and temperature can fluctuate by several degrees over the space of an hour (Biscoe et al., 1975a). Also much of the work in this area has been carried out on wheat only. There is a need for field experimentation looking at the mechanisms and processes controlling MGW in barley, in particular grain storage capacity.

Accurate determination of yield components is important. Harvesting techniques can put a limit on what is harvested as grain and what is separated as the chaff/straw portion. Loss of small grains from samples would impact upon the accuracy of MGW and grain number calculations and perhaps account for the apparent conservation of MGW. While small grains may not contribute much towards marketable yield in commercially grown crops, they will still have acted as a sink for assimilate produced by the crop, therefore for physiological investigations interested in source:sink balance these small grains must be captured in harvest data. Bloom (1985) has discussed how both combine-harvested and hand-harvested yield data can

be subject to bias – it is unclear which method is more appropriate. Either way, better understanding the morphology of these small grains may prove important in maximising yield potential and establishing source:sink relationships.

1.13 Experimental objectives

All experimentation as part of this thesis was carried out on spring-sown barley (spring barley). Spring barley accounts for approximately 85% of the total barley area in Ireland (Anon., 2011b) and as such results will be relevant and implementable on both a national and international scale. The experimental objectives were:

1. To determine the physiological basis of yield variation amongst spring barley crops across sites and years in Ireland
2. To identify the mechanisms underlying the determination of grain number in spring barley
3. To investigate potential trade-offs between grain number and grain weight in spring barley
4. To estimate the maximum yield potential of spring barley under Irish conditions

Chapter 2 **Determinants of spring barley yield in a high yield potential environment**

2.1 Introduction

The world population is increasing by 200 000 per day (Anon., 2011c). It has already broken the 7 billion mark and is estimated to reach over 10 billion by 2100. (Anon., 2012b) predicts a 60% increase in demand for agricultural production by 2050 and demand for cereals is projected to increase to 3 billion tonnes – a 43% increase from today's 2.1 billion tonnes (Anon., 2009). In terms of the world's most important crops by production quantity, barley (*Hordeum vulgare* L.) is ranked fourth amongst the cereals after maize, rice and wheat (Newton et al., 2011) and represents 60% of the land area devoted to cereal production in Ireland (Anon., 2011a). Optimising the performance of crops in areas of high yield potential is one possible approach to help meet the future increases in food demand whilst minimising global land use change.

Cereal production in Ireland is generally located, but not restricted to, the east, the south and the south east of the country where there are higher temperatures and solar radiation hours, and less precipitation than the west and north west. Based on data from the period 2000 to 2009, Ireland achieved the second highest yields of barley in the world at 6.6 t ha⁻¹ (Anon., 2011a). This is despite the fact that over 85% of the Irish barley crop is spring-sown barley (Anon., 2011b), which over the period 1985-2013 had a yield that was 82% that of that of winter-sown barley yield in Ireland (Anon., 2014). This further indicates the high yield potential of the temperate maritime Irish climate. Barley yield increases in Ireland of 20% and 28% on the previous decade were achieved in the 1970's and 1980's respectively (Anon., 2011a). This was most likely achieved through both improved breeding and improved agronomy (Abeledo et al., 2003; Bell et al., 1995; Slafer et al., 2005). Yield increases of 12% and 7% on the previous decade were achieved in the 1990's and 2000's respectively (Anon., 2011a) indicating that the rate of yield increase is slowing.

Yield of small grain crops is the product of two components – grain number and grain weight. Grain number in barley is highly correlated with yield across a range of

environments (Abeledo et al., 2003; Baethgen et al., 1995; Bingham et al., 2007a; Blake et al., 2006; del Moral et al., 2003; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013). Biomass production post-anthesis is a product of photosynthetically active radiation intercepted (PAR_{int}) and radiation use efficiency (RUE) during the period. Barley has the capability to produce a surplus of assimilate for grain filling from post-anthesis photosynthesis and pre-anthesis storage reserves (Bingham et al., 2007a; Gallagher et al., 1975; Habgood and Uddin, 1983; Wade and Froment, 2003). This has led to the conclusion that grain number largely determines and as such limits yield in barley and that further yield increases may be achieved through further grain number increases. The study environments that have established this strong grain number-yield relationship in barley include Argentina, Spain, Finland and the UK where average yields for the period 2000 to 2009 ranged from 2.8 t ha^{-1} to 5.8 t ha^{-1} (Anon., 2011a). It is unclear if grain number is a yield limiting factor in high yield potential climates such as Ireland and whether crops can produce enough assimilate to support high grain numbers.

If grain number remains a yield limiting factor in Ireland, it has not been established what physiological or morphological traits must be targeted in order to increase grain number. Grain number per unit area of barley is influenced by both ear number per unit area (Abeledo et al., 2003; Gallagher et al., 1975; Grausgruber et al., 2002), but also grain number per ear (Arisnabarreta and Miralles, 2008b; Gallagher et al., 1975). Initiation of both of these sub-components is largely completed before stem-extension (Kirby, 1977; Kirby and Appleyard, 1984).

The rapid ear growth period is crucial for grain number determination in wheat through its influence on grain number per ear (Abbate et al., 1997; Fischer, 1985; Miralles and Slafer, 2007; Reynolds et al., 2000). In barley, grain number per ear is similarly influenced by growth during the stem extension period (Grashoff and d'Antuono, 1997; Habgood and Uddin, 1983; Willey and Holliday, 1971). Some of the spikelets or florets initiated will die at a young age during stem elongation (Kirby, 1977; Kirby and Appleyard, 1984; Waddington and Cartwright, 1983) and prior to anthesis (Gallagher et al., 1975; Kirby, 1973). Stem-extension is a phase where there is a large increase in total crop growth rate (Kirby, 1977) and significant

spikelet mortality can occur due to a shortage of photosynthate (Arisnabarreta and Miralles, 2008a; Richards, 2000) and nitrogen (Baethgen et al., 1995) or in response to changes in environmental conditions, namely PAR interception (Fischer, 1985; Reynolds et al., 2009) and photoperiod (Gambín and Borrás, 2010). Grain number per ear is more conserved in barley than in wheat, in that wheat can compensate for low tiller numbers by increasing grain number per ear due to the high number of potentially fertile florets per spikelet in wheat (Wade and Froment, 2003). Barley has potential to alter grain number per ear, but not to the same extent as wheat because each barley spikelet contains only three florets (Wade and Froment, 2003). Further, the influence of grain number per ear on overall grain number per unit area may be less in two-row type barleys than in six-row types given that only one of the three florets is potentially fertile in two-row type barleys. Therefore the influence of ear number per unit area is likely to have a stronger bearing on overall grain number in two-row type barleys (Arisnabarreta and Miralles, 2008a).

The number of fertile ears is usually determined slightly before anthesis (Gallagher et al., 1975; Slafer et al., 2009). The traditional tillering pattern involves a rapid increase following the emergence of the third leaf on the main stem reaching a maximum around the beginning of stem extension as the crop moves into the floral initiation stage of development (del Moral et al., 1984). This is generally followed by a period of tiller death which largely occurs during stem extension until anthesis (del Moral et al., 1984; Gallagher et al., 1975) and then shoot number remains stable until harvest (del Moral et al., 1984; Gallagher et al., 1975; Slafer et al., 2009). A flush of late tillering is possible in response to a rainfall event following a period of drought (Jamieson et al., 1995) for example, but the contribution of these late tillers to yield is usually thought to be negligible (Kirby, 1967; Thorne and Wood, 1988). Similarly to spikelet mortality, competition for limited resources during stem extension can result in tiller mortality and as such reductions in the number of potential ears per unit area (Gallagher et al., 1975, 1976; Kirby, 1977). Around stem-extension, main shoot photoassimilate translocation shifts away from the tillers and towards the main stem itself (Lauer and Simmons, 1985) and unless tillers have reached a size and green area sufficient to independently produce the photoassimilates they require they may die (Kirby, 1977). Aside from the obvious influence of light quantity on the

amount of assimilate available per shoot, light quality may also influence shoot death. Reflection of far-red light from neighbouring plants has been shown to both reduce tiller production (Davis and Simmons, 1994; Skinner and Simmons, 1993) and promote tiller mortality (Sparkes et al., 2006). Also, nitrogen availability during stem-extension has been related to shoot mortality in barley (Wamser and Mundstock, 2007) and wheat (Sylvester-Bradley et al., 2001). There is a degree of overlap between the determination periods of ear number and grain number per ear. The same factors controlling grain number per ear may be responsible for the control of ear number. Also, the overlap of ear number and grain number per ear determination periods implies that there is a certain degree of trade-off between the two sub-components of grain number as they compete for the same limited supply of resources during the period. The relative importance of either sub-component for determination of overall grain number per unit area is unclear. The influence of ear number diminishes with higher seed rates due to reductions in the number of grains per ear (Wade and Froment, 2003; Willey and Holliday, 1971) but ear number is readily influenced by agronomic practices (Conry, 1995, 1998; Jenkyn et al., 1992; Wade and Froment, 2003). Understanding the relative importance and the dynamic that exists between the individual grain number components and resource availability will be important for tailoring agronomic practices aimed at increasing grain number.

The high yields in Ireland are achieved against a backdrop of high seasonal yield variability (Anon., 2012a). It is clear that environment x genotype interactions heavily influence any physiological crop study (Gallagher et al., 1983). The ratio of the amount of source (or energy captured and converted in the production of carbon assimilates by the plant to fuel growth) to sink (sink tissues are net importers of assimilates) will vary depending upon environmental conditions and season (Grashoff and d'Antuono, 1997; Serrago et al., 2013). Quantifying crop growth and development in commercial cereal crops of barley managed for high yield potential (Bingham et al., 2007a, b; Blake et al., 2006; Spink et al., 2000) across several sites and seasons using the same cultivar provides a dataset where the relative frequency of source or sink limitation to grain yield can be established. A detailed programme of assessments like this was carried out on spring barley across several sites and seasons in Ireland to aid the identification of factors that consistently limit yield. The

data provide a quantitative understanding of the yield-forming processes and help establish relationships between yield, yield components, and various measures of growth during standard developmental periods. The following hypotheses were tested:

- Grain number of spring-sown barley determines yield in the high yield potential Irish climate
- Grain number of a two-row spring-sown barley variety in Ireland is most readily influenced by ear number

2.2 Materials and Methods

The materials and methods described below are based on those of similar work carried out in the UK on winter barley by other workers (Bingham et al., 2007a; Blake et al., 2006).

For the purpose of this investigation and all further experimentation, a grain is defined as any vessel that has acted as a sink for post-anthesis assimilate. This may include small grains that are unmarketable in a commercial sense but for the purposes of physiological investigations on source and sink, must be included.

2.2.1 Experimental design and site characterisation

Six plots were marked out from commercially grown crops of spring barley at three sites (Oak Park, Co. Carlow (CW), Duncormick, Co. Wexford (WX) and Fermoy, Co. Cork (CK)) across three growing seasons (2011-13). Sites were located across the main arable cropping region in the east and south of Ireland. Sites were sheltered, relatively flat and in continuous arable rotations. Further site details are given in Table 2.1. Soil texture was identified using the method described by Tennyson et al. (2006). Topsoil samples (0 - 15 cm) were taken in each season for nutrient status analyses including phosphorous (P), potassium (K), pH, organic matter and micronutrients at all three sites. Meteorological data including daily rainfall; daily maximum, minimum and mean air temperature; total incident solar radiation; soil temperatures and humidity were obtained from national meteorological stations close to the three sites (maximum distance of 10 km). A high yield potential two-row spring barley (*Hordeum vulgare* L., cv. Quench) was the variety of choice due to its strong overall performance in the Irish Department of Agriculture, Food and the Marine (DAFM) Recommended Variety List.

*Table 2.1. Sowing date, latitude/longitude, altitude and soil properties for the three experimental sites: Oakpark, Co. Carlow (CW), Duncormick, Co. Wexford (WX) and Fermoy, Co. Cork (CK). * = missing data.*

Site/season	Sowing Date	Latitude, Longitude	Altitude (m)	Soil texture	Soil pH	Soil P (mg/l)	Soil K (mg/l)	Organic Matter (%)	Previous crop
CW 2011	10-Mar	52° 51' N, 6° 54' W	57	loam with moderate moisture holding capacity	7.65	39.18	150.91	5.8	winter barley
WX 2011	25-Mar	52° 14' N, 6° 39' W	24	clay loam with high moisture holding capacity	6.93	10.4	164.7	*	spring barley
CK 2011	16-Mar	52° 9' N, 8° 13' W	49	silt loam with moderate moisture holding capacity	6.4	9.7	57.6	*	winter wheat
CW 2012	14-Mar	52° 51' N, 6° 55' W	56	loam with moderate moisture holding capacity	6.78	5.72	105.3	*	winter wheat
WX 2012	03-Apr	52° 15' N, 6° 39' W	32	clay loam with high moisture holding capacity	*	*	*	*	spring barley
CK 2012	12-Mar	52° 9' N, 8° 13' W	50	silt loam with moderate moisture holding capacity	6.1	5.62	58.9	4.9	spring wheat
CW 2013	20-Mar	52° 51' N, 6° 54' W	59	loam with moderate moisture holding capacity	6.76	9.76	147.69	*	winter wheat
WX 2013	03-Apr	52° 15' N, 6° 42' W	41	clay loam with high moisture holding capacity	6.15	1.12	106	7.5	spring barley
CK 2013	04-Apr	52° 9' N, 8° 13' W	48	silt loam with moderate moisture holding capacity	6.1	7.2	44	4.2	spring barley

At each site, there were three plots for destructive sampling alternated with three plots for combine harvested yields within one bank of six plots. Plots were located within fields for even establishment and minimum interruption of commercially applied farm applications with as little as possible variation in soil related characteristics and avoiding serious weed problems. Plots were 4 m wide and ranged in length from 21 m to 24 m depending on the distance between the grower's tramlines (Figure 2.1). Combine harvested yields were taken from the centre 2.5 m of each plot.

Sites were managed for high yield potential with the aim of keeping the crop free of pests and disease using preventative measures. Nutrient applications were as per best practice outlined by Alexander et al. (2008). Macro- and micronutrient deficiencies were addressed with compound fertiliser applications pre-sowing and/or throughout the season. Nitrogen applications of 135-150 kg/ha were the maximum permitted in Nitrates Directive SI 610, 2010 factoring in the Nitrogen index of the soil and previous farm yields. Applications were split between early post-emergence when tramlines became visible and during tillering. Fungicides were applied shortly before stem extension and at ear emergence. All other applications of aphicide and herbicide were as required. Crops were sown at a rate of approximately 350 seeds/m² from early March to early April according to local conditions with the aim of achieving a plant stand of 250 – 300 plants/m².



Figure 2.1. A plot from the Cork site in 2011 shortly after stem-extension.

2.2.2 In-field assessments

The date of plant emergence was recorded as the first date on which the drilled rows could be clearly seen. Plant population counts were carried out at full crop emergence by counting the number of plants both sides of a 0.5 m marker at five locations per plot and converted to area based measurements using the row width. The crop was visited regularly (approximately weekly) from emergence onwards and checked for any pests, diseases, and weed infestation. Stages of plant development or crop growth stage (GS) were also recorded as per the Zadoks decimal scale outlined in Tottman (1987). For the nodal stages (GS 30 up to but not including GS 37) a date for a growth stage was recorded when more than half of main shoots reached the stage. From GS 37 onwards a plot was recorded as being of a specified stage when half of all shoots reached this point. Anthesis was judged to occur when half of the ear had emerged which corresponded to GS 55.

To determine the phyllochron, main stems of 10 plants were tagged before the onset of tillering in the plots designated for combine harvesting. Plants chosen were a minimum of 0.5 m from the ends and edges of plots, and from any tramlines or drill overlaps. From the beginning of leaf production (GS 12) until flag leaf emergence (GS 39) the number of main stem leaves were recorded. At each site visit new fully emerged leaves (ligule visible) were counted and a ring of light wire was placed above the youngest fully emerged leaf as a marker for the next assessment (Figure 2.2). The total number of potentially fertile shoots per plant was also recorded on the same 10 plants per plot until harvest. For this, an additional and larger wire ring was required on the soil around the base of the plant. This aided the identification of tillers from the observation plants as distinct from tillers from neighbouring plants (Figure 2.2). A shoot was counted when its prophyll or first leaf had emerged by 1 cm from its subtending leaf sheath. When counting total shoot number, primary, secondary and tertiary (if applicable) shoots were counted. These assessments were carried out at each site visit until physiological maturity and again at harvest.



Figure 2.2. Wire tag placed on the main stem above the youngest fully emerged leaf to enable leaf counts. Also shown is the larger wire tag at the base of the plant to enable shoot number per plant counts.

The visible presence of ear blight (associated with various *Fusarium* spp. (Osborne and Stein, 2007) in 2012 across the three sites prompted an in-field assessment of its severity during grain filling at all three sites when twenty-five ears per destructive sampling plot were assessed.

Radiation interception by the crop was determined at each site visit by simultaneously measuring radiation above and below the canopy using a Sunscan Canopy Analysis System (Figure 2.3, Delta-T Devices, Cambridge, UK) in the undisturbed plots designated for combine harvesting (Bingham et al., 2007b).



Figure 2.3. Hand held and fixed height ceptometers as part of the Sunscan Canopy Analysis System (Delta T Devices, Cambridge, UK) used to take light interception readings.

2.2.3 Destructive sampling

The plots for destructive sampling and subsequent growth analysis were sampled approximately weekly from emergence to physiological maturity with a further sample just prior to harvest (note: there was no pre-harvest growth analysis sample taken in CK in 2011). A quadrat sample size of 6 x 1 m adjacent row lengths of crop was removed from the field which equated to 0.72 m². There was at least 0.5 m distance between adjacent sample areas which were at least 0.5 m from the ends and edges of plots, and from any tramlines. Sample areas were representative of the plot and avoided drill overlaps. Samples were stored in sealed plastic bags in order to prevent drying out. If the subsequent growth analysis in the laboratory was delayed, samples were stored in a cold room at 4-6 °C. All growth analysis was generally completed within two days of sampling. If plants were contaminated with soil, it was removed by shaking or gently washing them under a running tap. All surface water was removed using paper towels or by shaking prior to growth analysis. Samples for dry matter determination were dried at 70 °C for 48 hours (or to a constant mass) and later finely ground (<2.0mm) in a cutting mill (RetschMühle, Retsch GmbH Haan, Germany) and analysed for total N with a Leco FP 328 autoanalyser (LECO

Corporation, St. Joseph, MI, USA). Projected green area was determined using a WD3 - WinDIAS Leaf Image Analysis System (Delta-T Devices, Cambridge, UK). The protocol for growth analysis varied depending on the growth stage.

Prior to GS31 whole plants (including roots) within the quadrat were sampled. A 75% sub-sample (by plant number) was obtained for estimation of above ground dry matter determination following removal of roots. Roots were also removed from the remaining 25% following plant and shoot number counts. Remaining material was then separated into four portions: green lamina; green stem and sheath; non-green or dead lamina, and non-green stem and sheath. Dead/dying shoots were removed from both sub-samples and their total fresh weight and dry weight recorded separately. A shoot was classified as dead/dying if it had no green material or its newest expanding leaf had begun to turn yellow at the tip (Laude et al., 1967). At later developmental stages a shoot which had its flag leaf fully emerged but with no evidence of booting or an ear was also classified as dead/dying. Lamina portions did not include the leaf sheath which remained in the stem and sheath portion. A leaf was classed as dead if it was yellow across the whole width of the base of the lamina. Yellowing parts of the leaves were classed as dead and if necrotic material was in isolated lesions a visual assessment of necrotic material was made and an equivalent portion removed from the end of the lamina. Individual fractions were then subject to dry matter determination and green area determination where applicable.

After GS31 plants within the selected quadrat area were cut at ground level with a sharp scissors or secateurs (roots not sampled). A 20% sub-sample (by shoot number) was obtained for above ground dry matter determination and further 10% was separated into the four portions as above with an additional two portions of green ear and awn and non-green ear and awn where applicable. Shoot counts were also carried out on this sub-sample. Subsequent analysis followed that of the pre-GS31 samples.

For the pre-harvest sample, plants within the selected quadrat area were cut at ground level with a sharp scissors or secateurs (roots not sampled). A 40% sub-sample (by shoot number) was obtained for above ground dry matter determination and further 20% was separated into ears and straw. Each portion was subject to dry matter

determination and ear counts were also carried out. Ears were then hand-threshed between two pieces of foam board (Figure 2.4) and sieved over a mechanically operated 1 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate into chaff and grain portions (Figure 2.5). Where chaff material remained in the grain portion on top of the sieve it was removed with tweezers and added to the chaff portion. Material was re-dried before the dry weight of each portion was recorded. MGW was also calculated for each plot using an automated grain counter (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate 25 g grain sample. After counting, grain weight was determined to the nearest 0.1 mg. Hand threshed grain yield (t ha^{-1}) was then expressed at 85 % dry matter, grain number m^{-2} calculated using MGW and plot yield data, and grain number per ear calculated using grain number data and ear number data. This yield data was retrospectively adjusted for the presence of non-grains as per section 3.2.1.4.



Figure 2.4. Ears were hand-threshed using the simple laboratory device shown



Figure 2.5. Chaff and grain portions resulting from the hand-threshing and sieving process

2.2.4 Combine harvesting and assessment of lodging

Lodging was assessed just prior to harvest or after a lodging event by estimating the proportion of the plot in each of the five categories: shoots upright; shoots leaning ($0-5^{\circ}$ from the vertical); shoots lodged ($5^{\circ}-45^{\circ}$ from the vertical); lodged and flat ($45^{\circ}-90^{\circ}$ from the vertical); brackled (stem failure of $>1/4$ or more up its length).

At final harvest, all plots designated for combine harvesting were harvested using a plot harvester. Three different plot harvester models were used (Class Dominator 38, Class GmbH, Germany; Deutz Farmliner 3370, Deutz AG, Cologne; Sampo 2010, Sampo-Rosenlew Ltd., Finland) depending on site and season. Grain from each plot was weighed by the harvester independently and a sample was taken for moisture content and mean grain weight (MGW) calculations. Grain yield and its components were then calculated as described for the pre-harvest sample. Combine threshing and separation apparatus were set up with the objective of achieving a clean sample while losing as little grain as possible.

2.2.5 Further analysis of data

2.2.5.1 *Phyllochron*

Phyllochron was calculated by plotting the number of emerged leaves on the main stem against thermal time from the first assessment. Thermal time was calculated (for this and other assessments) with a base temperature of 0 °C and as per method 1 of McMaster and Wilhelm (1997) below which it is assumed no development occurred (Frank and Bauer, 1995; Kirby, 1995; McMaster et al., 2003; Miralles et al., 2001; Paynter et al., 2004). A simple linear regression was carried out on the data from each site using GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK) and the phyllochron was then estimated as the reciprocal of the gradient of the fitted line as per (Blake et al., 2006).

2.2.5.2 *Total biomass and ear biomass*

Total biomass and ear weight data were plotted against thermal time from sowing at each site/season. Ear weight was used as a proxy for grain weight because, after anthesis, ear dry weight increase is almost entirely accounted for by the filling of the grains (Gallagher et al., 1975). The start and end of the periods of rapid linear growth were estimated from logistic regressions fitted to these plots using GenStat. These time points were determined according to Bingham et al. (2007b) where the equation of the curve was differentiated with respect to time to give the instantaneous rate of biomass or ear growth. The start and end points of the rapid linear growth period were then identified as the points at which the percentage change in rate in the accelerating and decelerating phases (either side of the linear phase) were minimized. The dates and developmental stages at which these points occurred were calculated.

2.2.5.3 *Green area index (GAI)*

Polynomial regressions (2nd order) were fitted to plots of GAI against thermal time from sowing for each site/season using Microsoft Excel (Microsoft Office 2010, Microsoft Corporation, Washington) to determine maximum GAI values and

estimate green area duration post-anthesis. Early season data points (pre-GS 31) and late season data points (< 0.5 GAI) were omitted from the plots in order to remove ‘tails’ and enable a better fit for the polynomial curve at the maximum. Post-anthesis green area duration was estimated as the area under the graph from GS 55 to senescence.

2.2.5.4 Radiation Use Efficiency (RUE)

To estimate pre-anthesis PAR_{int} the transmitted (below canopy) and incident (above canopy) radiation values were used to calculate the fraction of radiation intercepted by the canopy and interpolated for the days in between each sample date. The daily total incident radiation value from the nearby meteorological station was then used to calculate daily PAR_{int} . PAR was estimated as $0.5 \times$ solar radiation (McCree, 1981).

Post-anthesis PAR_{int} by healthy green tissue was estimated using green area data from destructive samples and an extinction co-efficient (k) calculated at anthesis (Bingham et al., 2007a). Daily PAR_{int} was then calculated as described for pre-anthesis time points. Cumulative values between each growth analysis sample date were then plotted against cumulative dry matter gain values.

Radiation use efficiency RUE ($g\ MJ^{-1}$) was estimated from linear regressions of accumulated intercepted photosynthetically active radiation (PAR_{int}) versus accumulated dry matter plotted for each site/season from GS31 onwards where biomass began to accumulate at a rapid rate. Both split-line and single line regressions were carried out on these data using GenStat to investigate best fit and whether RUE declined or levelled-off post-anthesis. Where significant non-linearity of these plots occurred post-anthesis, maximum post-anthesis RUE was estimated from the initial linear portion of the regression (Bingham et al., 2007a).

Where an actual post-anthesis value of RUE was required linear regressions were fitted to data points from GS55 onwards and RUE was calculated as above. As the data set for each site/season was limited to 5-8 data points in this instance it was not possible to fit split line regressions therefore the data were treated as linear.

2.2.5.5 Yield data

Yield, grain number m^{-2} and MGW along with grain number per ear and shoot number m^{-2} data were calculated from the pre-harvest growth analysis sample. However at CK 2011 it was not possible to take a pre-harvest growth analysis sample so equivalent hand-threshed yield component values were estimated from the combine-threshed grain sample (using the equation of the line from linear regression analysis of combine-threshed values versus hand-threshed values for the other 8 site/seasons). Ear number m^{-2} values at harvest were estimated by using values from the previous growth analysis sample. These estimated values for CK 2011 are marked with an asterisk or displayed as open symbols in the tables and charts below.

2.2.5.6 Season and site effects

Yield, yield component and maximum leaf number per main stem values were assessed for site, season, and site x season effects using a general ANOVA treatment structure with GenStat. The model was as follows: season + site + season.site + site/block. Block was nested in site to account for the fact that blocks at one site were distinct from blocks at another site. Ear blight data for the sites in 2012 was also assessed for site effects in this way.

A similar model was used to test whether differences between mid-season minimum shoot numbers and harvest ear numbers across sites and seasons were significant. An additional timing factor with two levels was added (minimum or harvest). The model was as follows: season + site + timing + season.site.timing + site/block.

Following ANOVA, relevant means for treatments of interest were compared using the standard error of the difference (S.E.D.) between means, on the residual degrees of freedom (d.f.) from the ANOVA, thus invoking the least significant difference (L.S.D.) at the $P = 0.05$ level of significance.

2.2.5.7 Further correlation and linear regression analysis

To test the hypothesis that the chances of a shoot surviving to form an ear is related to its size and potential to capture resources at the start of stem extension, various measures of shoot weight, size and N content at GS 31 along with other measures of growth before and during stem-extension were correlated with shoot survival from the early season maximum shoot number to the identified mid-season minimum shoot number for all site/seasons except CW 2013 for reasons discussed later.

To investigate the mechanisms by which crops of higher grain number/m² are able to meet the grain demand for dry matter, linear regression analysis was carried out on plots of grain number m⁻² against several post-anthesis variables for all 9 site/seasons of data. Without an estimate of stem carbohydrate storage reserves at anthesis, stem biomass and its decline from anthesis to physiological maturity (GS 55 – GS 87) was used as a proxy for stem reserve utilisation during grain filling.

2.3 Results

2.3.1 Climatic conditions

Temperature, solar radiation and rainfall data for each site/season along with average values throughout the spring barley growing season (March – August) are given in Figure 2.6, Figure 2.7, and Figure 2.8. The 2011 season was warmer than average at the start and cooler than average towards the end. The opposite was true for 2013. Temperatures in 2012 were slightly warmer than average in March but comparable to or cooler than average for the other months. There was little difference between sites within a given season. The highest monthly accumulated solar radiation levels of the three seasons were in 2013. In 2011 solar radiation was close to and sometimes slightly above average while in 2012 it was well below average particularly in June and July. Again, there was relatively little difference in the pattern between sites in any given year except for WX 2013 which received slightly higher levels of solar radiation during the period May to July than the other two sites in that year. The accumulated monthly rainfall data tended to reflect the pattern of solar radiation where high rainfall levels accompanied low solar radiation levels and vice versa. There were very high levels of rainfall in the summer of 2012, particularly in June. The 2013 season had the wettest start while 2011 had the driest however in both cases, across the entire growing season total rainfall values remained below or close to the long-term average.

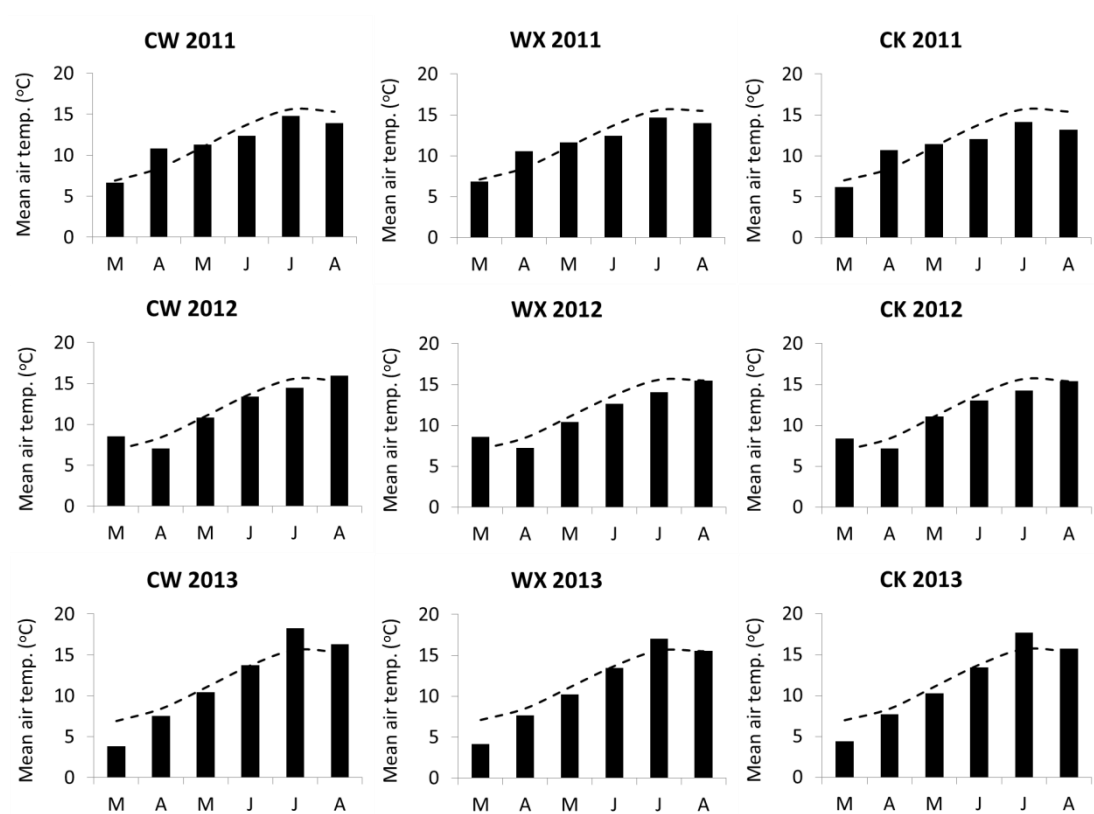


Figure 2.6. Monthly mean temperatures ($^{\circ}\text{C}$) from March to August at all site/seasons. Associated long-term-average values (1981-2010) for each site are shown as a broken line.

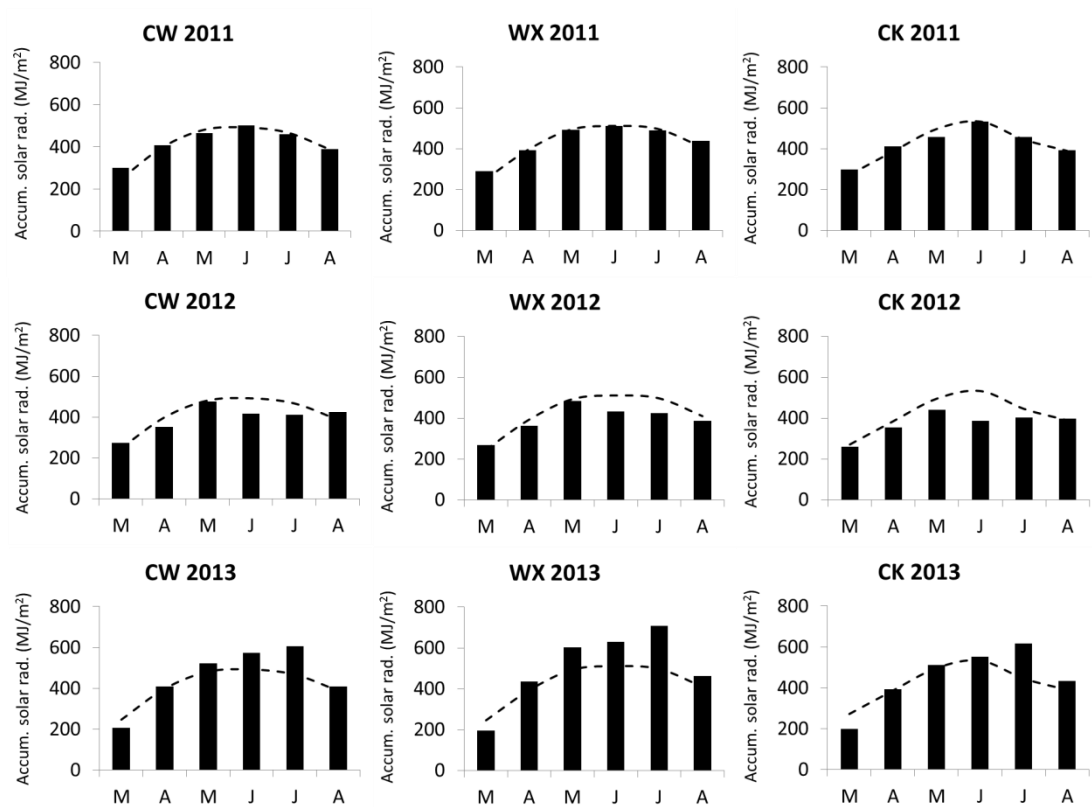


Figure 2.7. Monthly accumulated solar radiation (MJ/m^2) from March to August at all site/seasons. Associated long-term-average values (2005-2012 for CW and WX; 2008-2010 for CK) for each site are shown as a broken line.

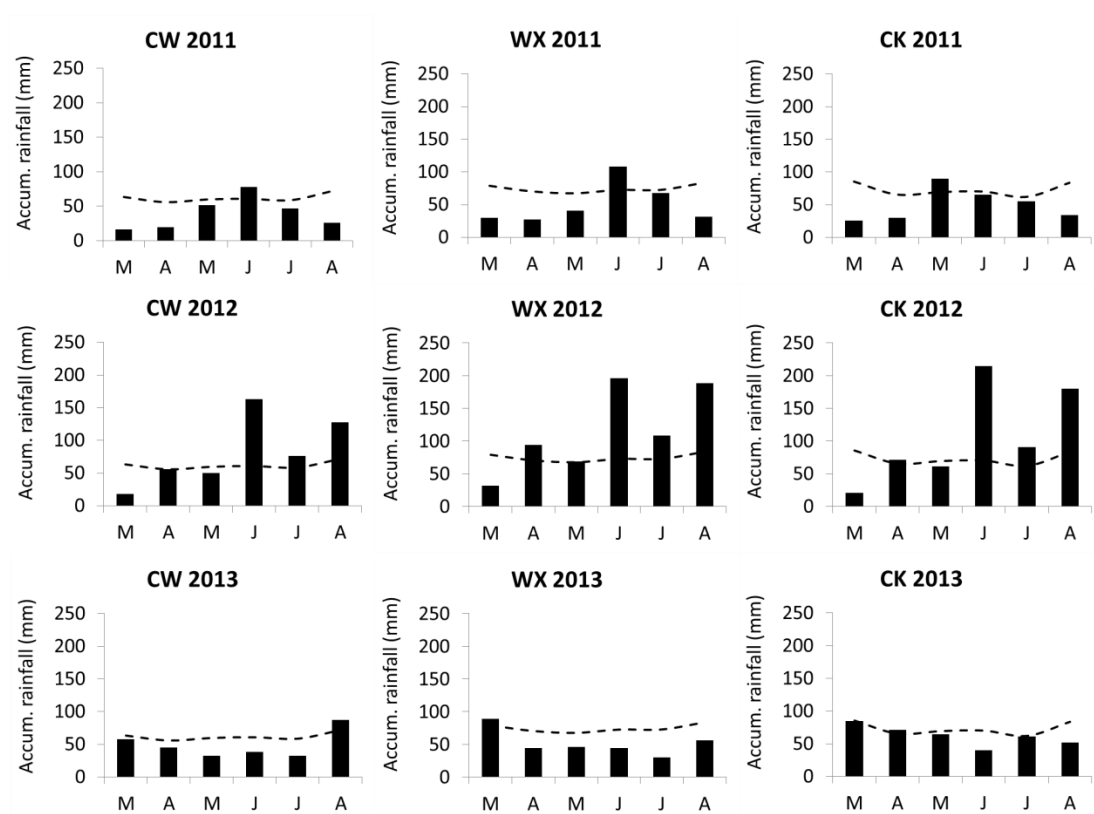


Figure 2.8. Monthly accumulated rainfall (mm) from March to August at all site/seasons. Associated long-term-average values (1981-2010) for each site are shown as a broken line.

2.3.2 Yield and yield components

Yield and yield component values are given in Table 2.2. Higher yields were achieved in 2011 than in 2012 and 2013 ($P < 0.001$). There was a significant site effect on yield ($P = 0.006$) and a close to significant season x site interaction ($P = 0.053$), with CW achieving the lowest or joint lowest of the three sites in all three seasons and CK achieving the highest in two out of three seasons. A higher grain number m^{-2} was also achieved in 2011 than in 2012 and 2013 ($P < 0.001$) with CK consistently achieving the highest of the three sites followed by WX and CW ($P < 0.001$). Grain number m^{-2} at CW in 2013 was particularly low in comparison to other sites in that season. MGW in 2012 was lower than in 2011 and 2013 ($P < 0.001$). There was a significant season x site interaction effect on ear number m^{-2} ($P = 0.009$) where WX achieved the highest in 2011 and 2013 but not in 2012. As with grain number m^{-2} , ear number m^{-2} at CW in 2013 was particularly low in comparison to other sites in that season. There was a close to significant site effect on grain number per ear ($P = 0.056$) where CK achieved the highest of the three sites in 2011 and 2013 but not in 2012.

Table 2.2. Yield and yield components for all site/seasons. Season means in bold. * = CK harvest values estimated from combine samples and/or previous growth analysis sample. P values and L.S.D.'s at 5% for season, site, and season x site interaction effects are also given. Yield and mean grain weight (MGW) values are expressed at 85% dry matter.

Means	Yield (t ha ⁻¹)	Grain no. m ⁻²	MGW (mg)	Ear no. m ⁻²	Grain no. ear ⁻¹
CW 2011	9.50	19323	49.26	1057	18.32
WX 2011	10.38	21524	48.30	1205	17.91
CK 2011	10.65*	23317*	45.69*	1038*	22.46*
2011 mean	10.18	21388	47.75	1100	19.57
CW 2012	7.24	17226	41.96	819	21.02
WX 2012	7.24	17276	41.97	913	19.09
CK 2012	9.21	21174	43.40	1114	19.03
2012 mean	7.89	18559	42.44	949	19.71
CW 2013	5.49	10948	50.17	664	16.52
WX 2013	9.13	17430	52.45	973	17.95
CK 2013	7.85	17550	44.47	870	20.18
2013 mean	7.49	15310	49.03	836	18.22
<i>Grand mean</i>	<i>8.52</i>	<i>18419</i>	<i>46.41</i>	<i>961</i>	<i>19.17</i>
Effects					
<i>Season P-value</i>	<i><.001</i>	<i><.001</i>	<i><.001</i>	<i><.001</i>	<i>0.235</i>
<i>L.S.D. 5%</i>	<i>1.046</i>	<i>1901.7</i>	<i>2.84</i>	<i>85.5</i>	<i>ns</i>
<i>Site P-value</i>	<i>0.006</i>	<i><.001</i>	<i>0.077</i>	<i>0.001</i>	<i>0.056</i>
<i>L.S.D. 5%</i>	<i>1.046</i>	<i>1901.7</i>	<i>ns</i>	<i>85.5</i>	<i>2.07</i>
<i>Season x site P-value</i>	<i>0.053</i>	<i>0.105</i>	<i>0.097</i>	<i>0.009</i>	<i>0.084</i>
<i>L.S.D. 5%</i>	<i>1.812</i>	<i>ns</i>	<i>ns</i>	<i>148.2</i>	<i>ns</i>

2.3.3 Lodging and ear blight

Lodging and brackling occurred only at WX and CK in 2013, however, no stems were lodged flat (45°-90°) at either site and the lodging and brackling described occurred after physiological maturity therefore would not have impacted upon grain filling. Plot scores of 70% and 47% for stem lodging (5°-45°) and 15% and 38% for brackling were recorded at WX and CK respectively. Yield loss was expected to be minimal, especially from hand-harvested quadrats.

The percentages of ear area with symptoms of ear blight at CW, WX and CK in 2012 were 23%, 10% and 0.09% respectively. CW in 2012 had significantly higher levels than WX and CK in 2012 ($P < 0.001$, L.S.D. 6.2).

2.3.4 Crop growth, development, and resource capture

Crop development at the three sites followed a largely similar trend within seasons. Across all site/seasons, a range in sowing date of 25 days was reduced to a range in harvest date of 19 days. Table 2.3 shows a mean plant number m^{-2} of 272 was achieved across all site/seasons equating to an establishment rate of 78%; plant establishment was particularly low at CW in 2013.

The mean leaf number per main stem achieved for the 9 site/seasons was 8.2 (Table 2.3) with a significant site x season interaction effect ($P = 0.027$, L.S.D. 5% 0.55, range 0.8). The mean phyllochron for the 9 site/seasons was estimated at 82 °C days (range 19.7) (Table 2.3). A grouped simple linear regression was performed on the leaf emergence and thermal time data using GenStat and this showed that slopes (the reciprocal of which was the phyllochron) did not differ significantly between sites ($P = 0.323$), seasons ($P = 0.303$) and site/season combinations ($P = 0.141$).

Table 2.3. Measures of growth, development and resource capture for all 9 site/seasons. Season means in bold. Grand mean and SEM in italics. GAI = green area index; GAD = green area duration post-anthesis; RUE = radiation use efficiency.

Site/ season	Plant no. m ⁻²	Duration of rapid total biomass growth (days)	Rate of rapid total biomass growth (t ha ⁻¹ day ⁻¹)	Duration of rapid ear growth (days)	Rate of rapid ear growth (g ear ⁻¹ day ⁻¹)	Duration sowing - GS31 (days)	Duration GS31 - GS55 (days)	Duration GS55 - GS87 (days)	Duration sowing - harvest (days)	Max leaf number per main stem	Phyllochron (°C days)	Max GAI	GAD (GAI days)	RUE (g MJ ⁻¹)
2011														
CW	269	35	0.225	25	0.020	60	28	55	160	8.4	77.8	4.1	2082	2.35
WX	257	33	0.278	28	0.018	46	28	50	159	8.3	72.8	5.3	2472	3.04
CK	312	39	0.234	21	0.025	56	27	49	161	7.9	86.1	6.6	2674	2.83
mean	279	36	0.246	25	0.021	54	28	51	160	8.2	78.9	5.3	2409	2.74
2012														
CW	321	45	0.178	19	0.025	68	29	49	160	8.2	81.9	5.9	2695	2.56
WX	233	30	0.260	25	0.022	58	31	43	147	8.6	80.1	5.3	1821	3.05
CK	347	49	0.181	24	0.020	71	30	42	163	8.0	87.6	6.4	2784	2.89
mean	300	41	0.206	23	0.022	66	30	45	157	8.3	83.2	5.9	2434	2.83
2013														
CW	183	24	0.226	11	0.038	70	26	37	145	7.8	84.4	3.4	1731	2.73
WX	280	31	0.270	14	0.039	55	26	38	140	7.9	73.4	5.7	1800	2.37
CK	246	33	0.235	12	0.038	53	32	32	139	8.5	92.5	6.6	2056	2.40
mean	236	29	0.244	12	0.038	59	28	36	141	8.1	83.4	5.2	1863	2.50
<i>Grand mean</i>	<i>272</i>	<i>35</i>	<i>0.232</i>	<i>20</i>	<i>0.027</i>	<i>60</i>	<i>29</i>	<i>44</i>	<i>153</i>	<i>8.2</i>	<i>81.8</i>	<i>5.5</i>	<i>2236</i>	<i>2.69</i>
<i>SEM</i>	<i>16.7</i>	<i>2.6</i>	<i>0.012</i>	<i>2.1</i>	<i>0.003</i>	<i>2.8</i>	<i>0.7</i>	<i>2.5</i>	<i>3.3</i>	<i>0.1</i>	<i>2.2</i>	<i>0.37</i>	<i>141</i>	<i>0.09</i>

To investigate the variation in rates and duration of crop growth between site/seasons logistic functions were fitted to plots of total above ground biomass and ear biomass against thermal time from sowing (Figure 2.9; Figure 2.10). The logistic regression model fitted the data well with R^2 values exceeding 0.95 for both variables at all site/seasons (P always < 0.001). The beginning and end points of the rapid linear growth phases were estimated. Total biomass growth accelerated around GS31 but began to slow down before total ear growth at all site seasons. There was on average a 14 day period between the estimated point at which total biomass growth slowed and ear growth slowed meaning ear dry matter accumulation continued at a rapid rate while total biomass accumulation began to slow down.

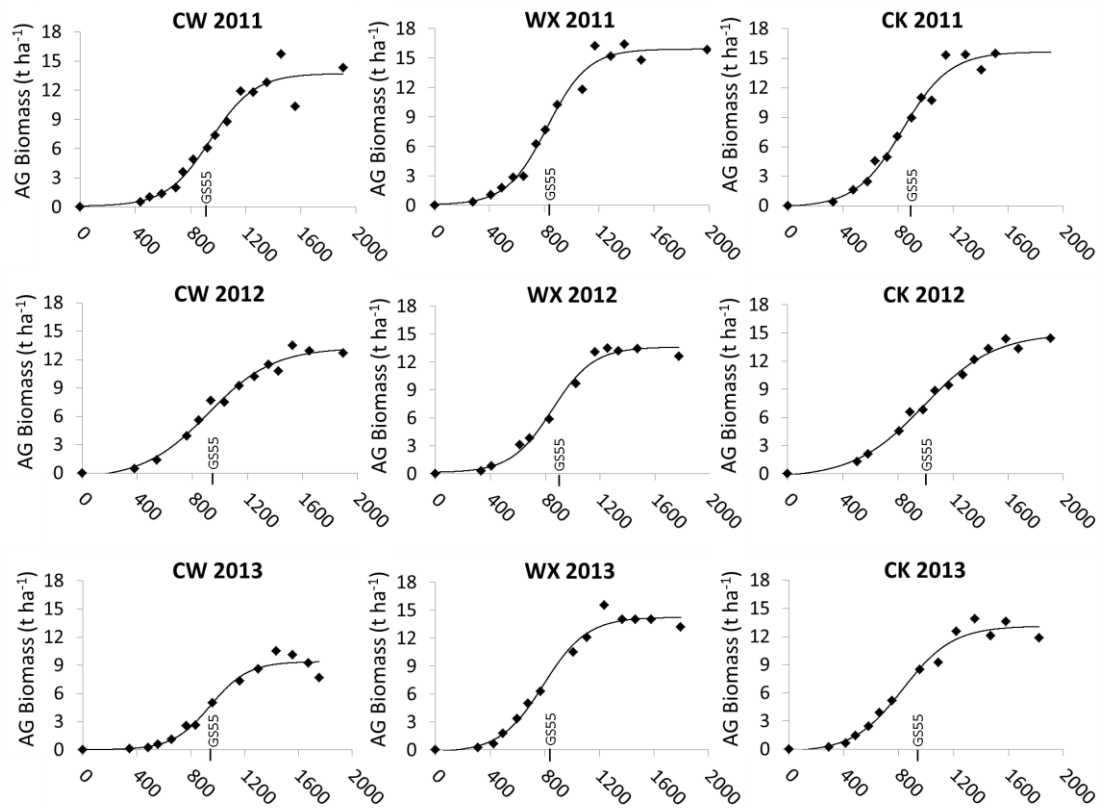


Figure 2.9. Plot of thermal time from sowing ($^{\circ}\text{C}$ days) on the x-axis versus total above ground (AG) biomass (t ha^{-1} at 100% dry matter) on the y-axis for all site/seasons. Plots are fitted with a logistic regression. Zadoks GS55 (anthesis) is marked. Each point is the mean of three replicates.

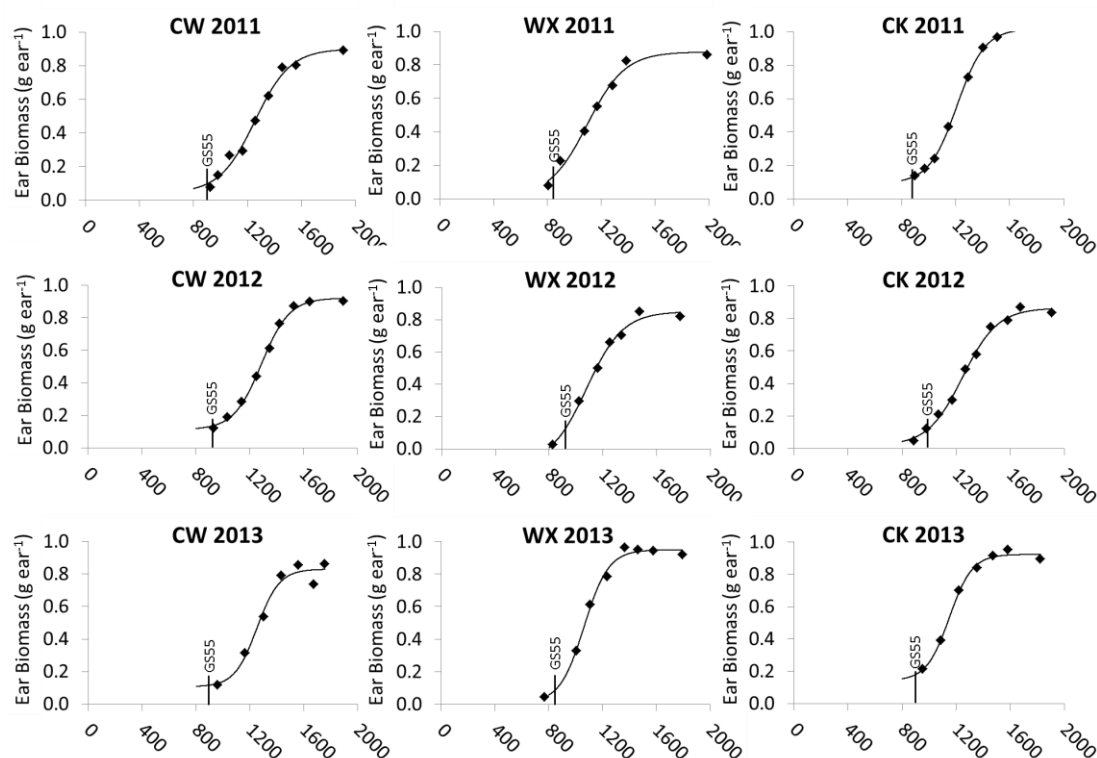


Figure 2.10. Plot of thermal time from sowing ($^{\circ}\text{C}$ days) on the x-axis versus ear biomass (g ear^{-1} at 100% dry matter) on the y-axis for all site/seasons. Plots are fitted with a logistic regression. Zadoks GS55 (anthesis) is marked. Each point is the mean of three replicates.

The estimated start of the period of rapid linear total biomass growth occurred on average 69 days (range 28) and 647 $^{\circ}\text{C}$ days (range 181) after sowing. The estimated start of the period of rapid grain growth occurred on average 11 days (range 18) and 138 $^{\circ}\text{C}$ days (range 248) after anthesis. Total biomass growth and grain growth continued beyond the end of the estimated rapid period but at lower rates. The estimated durations and rates of these linear phases of rapid growth are shown in Table 2.3.

Polynomial regressions (2nd order) fitted plots of thermal time versus GAI reasonably well with R^2 values in excess of 0.74 at all site/seasons (Figure 2.11). The estimated max GAI occurred on average 62 $^{\circ}\text{C}$ days (range 242) after anthesis. This is understandable as anthesis was adjudged to occur when the ear was half emerged

(GS 55) so maximum GAI would not be expected to occur until slightly later when the stem and in particular the peduncle complete extension. Max GAI ranged in value from 3.4 at CW 2013 to 6.6 at CK in 2011 and 2013 and green area duration post-anthesis (GAD) ranged in value from 1731 GAI days at CW 2013 to 2784 GAI days at CK 2012 (Table 2.3).

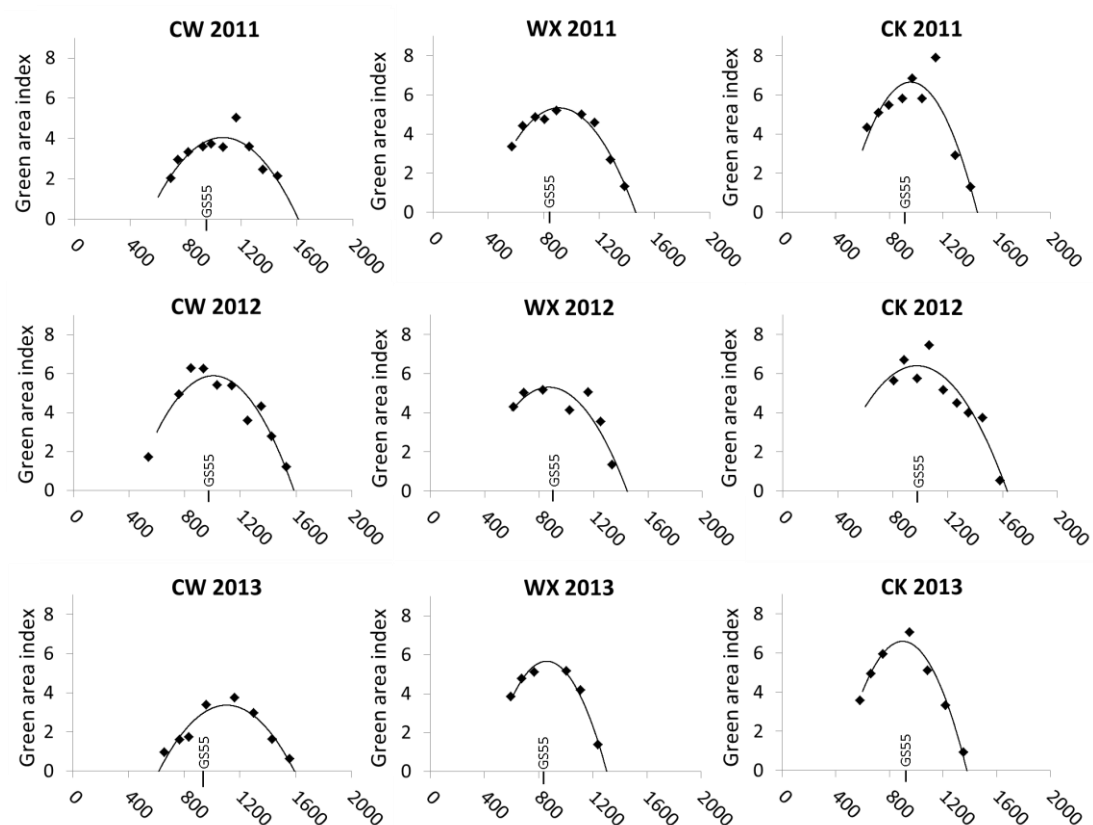


Figure 2.11. Polynomial (2nd order) regressions fitted to plots of thermal time on the x-axis (°C days) versus green area index (GAI) on the y-axis for each site/season

All sites except CW 2013 intercepted greater than 93 % of available solar radiation at anthesis. A value of just 70 % was achieved at CW 2013 where canopy closure did not occur due to the poor and delayed crop establishment.

Significant non-linearity of RUE occurred at WX 2011 and CK 2011. At these two site/seasons a split line (two-line) regression accounted for more of the variation than a single line regression (R^2 values increased from 0.96 to 0.98 at WX 2011 and from

0.92 to 0.96 at CK 2011). The slope of the second line was not significantly different to zero, therefore it was assumed RUE leveled-off. Here, maximum post-anthesis RUE was estimated as the slope of the first line, which covered the developmental period from stem extension (GS 31) to approximately early dough (GS 83) at the two sites. At all other site/seasons RUE was linear where regression analysis gave R^2 values in excess of 0.89 and P always <0.001 . Data are displayed in Figure 2.12 and Table 2.3. A grouped simple linear regression was performed on the intercepted PAR and accumulated dry matter gain data from the linear portions using GenStat and this showed that slopes (RUE) did not differ significantly between sites ($P = 0.860$). However there was a close to significant difference between seasons ($P = 0.051$) and a significant difference between site/season combinations ($P = 0.028$).

When post-anthesis data points only were used to estimate post-anthesis RUE at all 9 site/seasons, linear regression while significant at each site/season was statistically weaker than season-long estimates ($0.001 < P < 0.071$; $0.62 < R^2 < 0.96$). A grouped simple linear regression was performed on the intercepted PAR and accumulated dry matter gain post-anthesis data using GenStat and this showed that slopes (RUE) did not differ significantly between sites ($P = 0.592$), seasons ($P = 0.403$) and site/season combinations ($P = 0.738$).

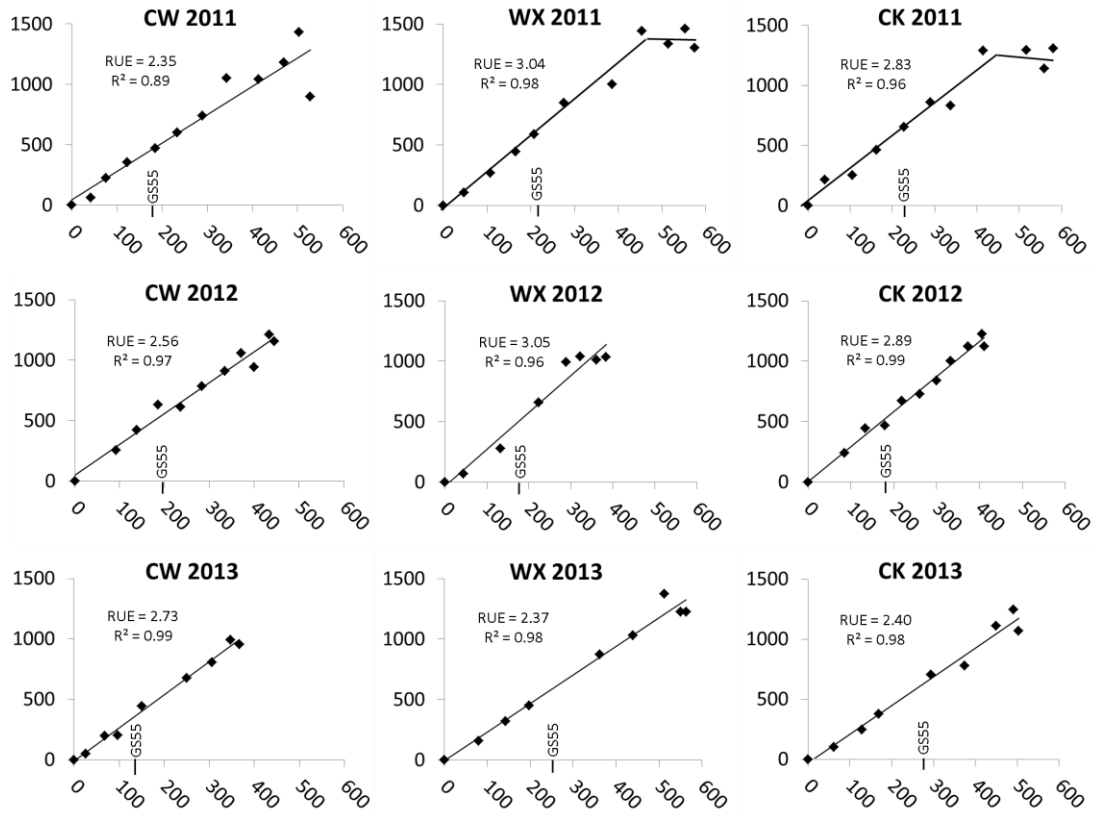


Figure 2.12. Regression analysis for plots of accumulated PAR intercepted (MJ m^{-2}) on the x-axis versus accumulated dry matter gain (g m^{-2}) on the y-axis at all site seasons from GS 31 to senescence. Radiation use efficiency (RUE, g MJ^{-1}) was estimated as the slope of the line. Split-line regression is shown for WX 2011 and CK 2011. GS55 (anthesis) is marked.

2.3.5 Determinants of yield

Of the two main yield components, grain number m^{-2} accounted for most of the variation in yield ($P < 0.001$, $R^2 = 0.84$), while MGW remained relatively more conserved across sites and seasons (see Figure 2.13 (a) and (b)). In turn, ear number m^{-2} accounted for most of the variation in grain number m^{-2} ($P = 0.002$, $R^2 = 0.75$) while grain number per ear remained relatively more conserved across sites and seasons (see Figure 2.13 (c) and (d)). There was no relationship between ear number m^{-2} and grain number per ear ($P = 0.473$).

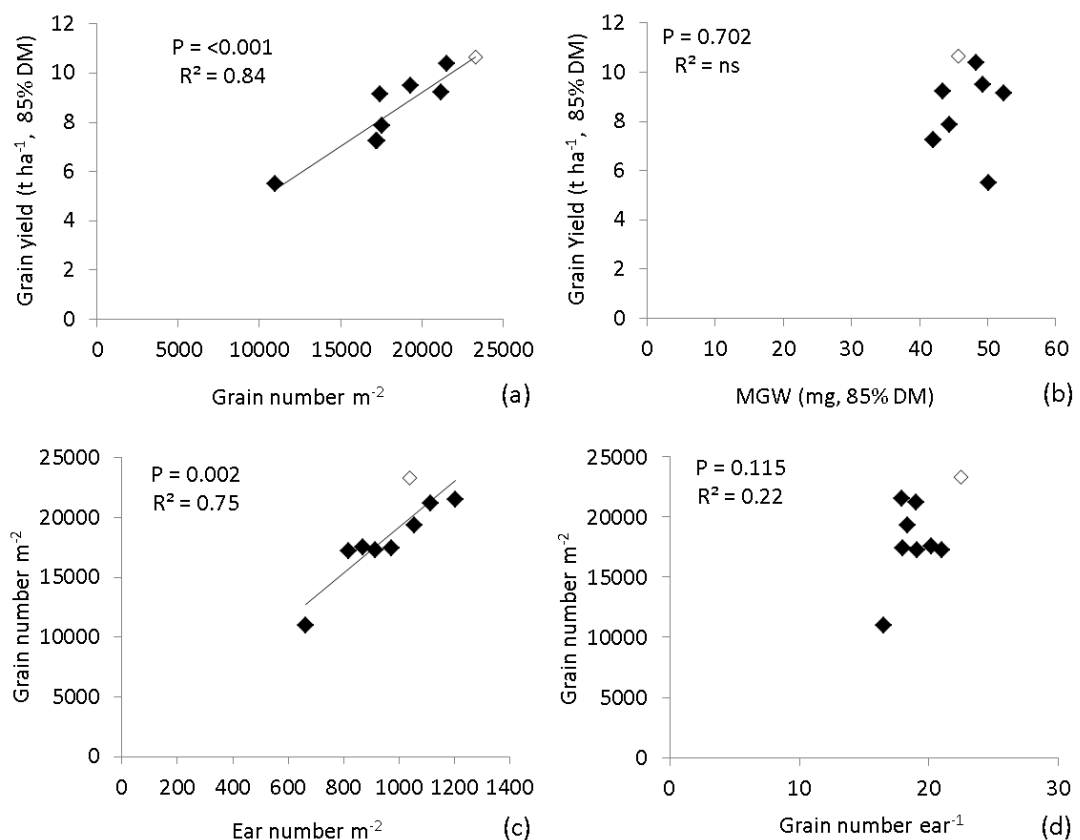


Figure 2.13. Linear regressions of (a) grain number m^{-2} versus yield; (b) mean grain weight (MGW) versus yield; (c) ear number m^{-2} versus grain number m^{-2} and (d) grain number per ear versus grain number m^{-2} . Hand-threshed data is used. Unfilled marker is the CK 2011 data point where values were estimated from combine data. DM = 100% dry matter.

2.3.6 Tillering dynamics

Shoot number m^{-2} was plotted against thermal time for each site/season and is shown in Figure 2.14. The tillering pattern widely reported in the literature of maximum production at the beginning of stem-extension (GS31), followed by a period of tiller death up to anthesis (GS55) then stabilisation through to harvest was not clearly evident across sites and seasons. An early-season maximum shoot number occurred at or around GS31 at 6 of the nine site/seasons, between GS31 and GS55 at two site/seasons and after flowering in one site in one season (CW 2013). A period of early-mid season shoot death has been identified which, while not strictly occurring from GS31 to GS55, began during the stem-extension period in all site/seasons except CW 2013 but continued after flowering in 5 of the site/seasons. This period of shoot death was followed by some degree of post-anthesis re-tillering which was itself followed by further death and apparent re-tillering at some site/seasons. The data presented in Figure 2.14 were taken from quadrat samples. Quadrats represent a limited area of the plot whose location differs between different time points. Variation in crop growth within the plot might contribute to variation in shoot numbers over time as illustrated by the relatively large error bars at some time points in Figure 2.14. Revisiting and closely inspecting the same set of tagged plants weekly for shoot growth and death provided a potentially more accurate measure of tillering dynamics than bulk quadrat samples. Figure 2.15 shows a broadly similar tillering pattern for all site/seasons albeit in shoot number per plant format, to that shown in Figure 2.14. Thus the time at which the early-season maximum shoot number was achieved and subsequent duration of shoot mortality were comparable. There was also similar evidence of subsequent re-tillering post-anthesis. The largely similar patterns observed between Figure 2.14 and Figure 2.15 provide confidence that the maxima and minima shoot number described above are real events resulting from tiller production, mortality and re-tillering and not a consequence of sampling error. Excluding CW 2013, the mean early season maximum value at the start of the identified shoot death period was 1212 shoots m^{-2} (range 233), the mean mid-season minimum value was 854 shoots m^{-2} (range 250) and the mean harvest value was 999 shoots m^{-2} (range 386).

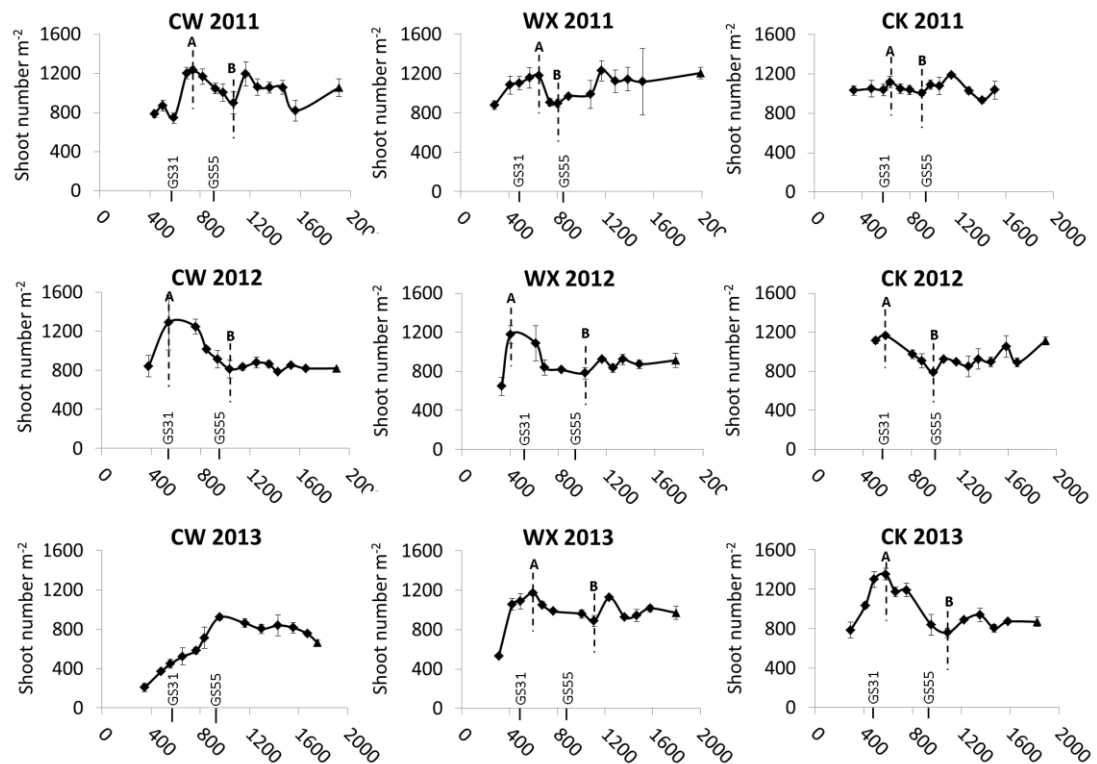


Figure 2.14. Shoot number m^{-2} data (y-axis) from quadrat samples for each site/season plotted against thermal time from sowing (x-axis). Vertical dashed lines indicate the period of early-mid season shoot death where A = early season maximum shoot number m^{-2} and B = mid-season minimum shoot number m^{-2} . Zadoks growth stages GS 31 (beginning of stem-extension) and GS 55 (50% ear emergence taken to coincide with anthesis) are labelled. Error bars are + one standard error of the mean. Values include both ear-bearing and non-ear-bearing potentially fertile tillers except at harvest where only ear-bearing tillers were counted (\blacktriangle). No harvest value available for CK 2011.

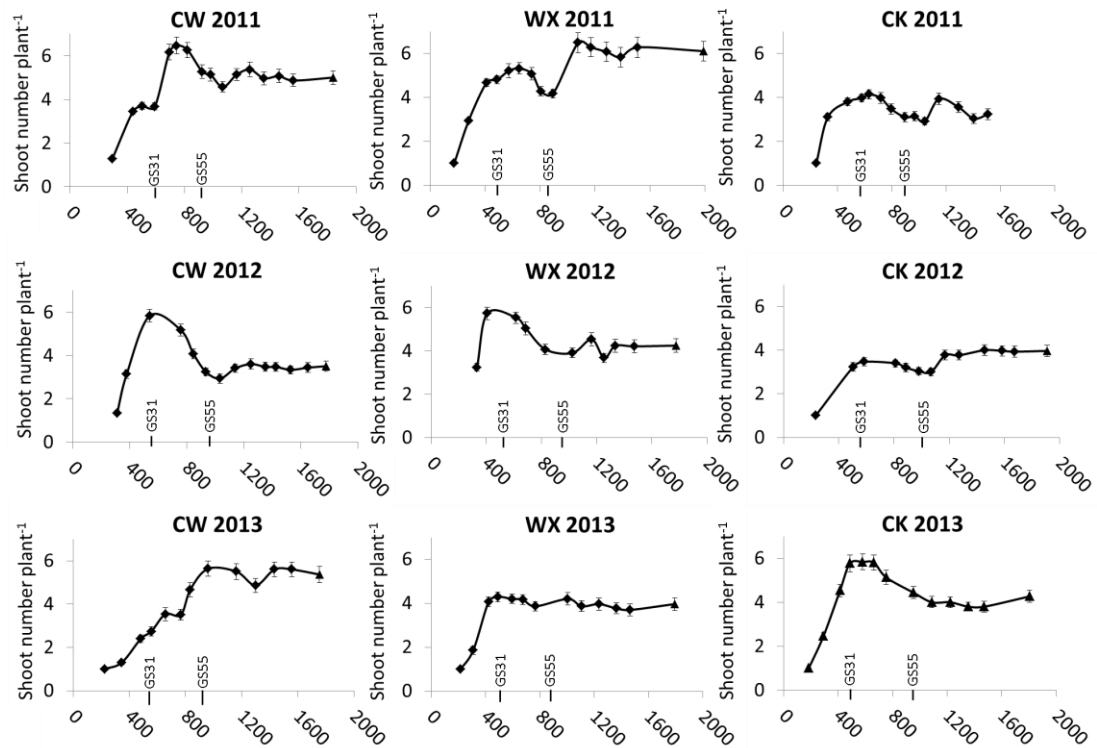


Figure 2.15. Shoot number per plant data (y-axis) from tagged plants for each site/season plotted against thermal time from sowing (x-axis). Zadoks growth stages GS 31 (beginning of stem-extension) and GS 55 (50% ear emergence taken to coincide with anthesis) are labelled. Error bars are + one standard error of the mean. Values include both ear-bearing and non-ear-bearing potentially fertile tillers except at harvest where only ear-bearing tillers were counted (▲). No harvest value available for CK 2011.

The results of correlation analysis show that when CW 2013 data is excluded, early season maximum shoot number m^{-2} had a weak relationship with harvest ear number m^{-2} (Figure 2.16 (a), $P = 0.109$, $r = 0.52$) across sites and seasons. On the other hand, there was a strong positive correlation between % shoot survival from this early season maximum through to harvest and harvest ear number m^{-2} (Figure 2.16 (b), $P < 0.001$, $r = 0.96$).

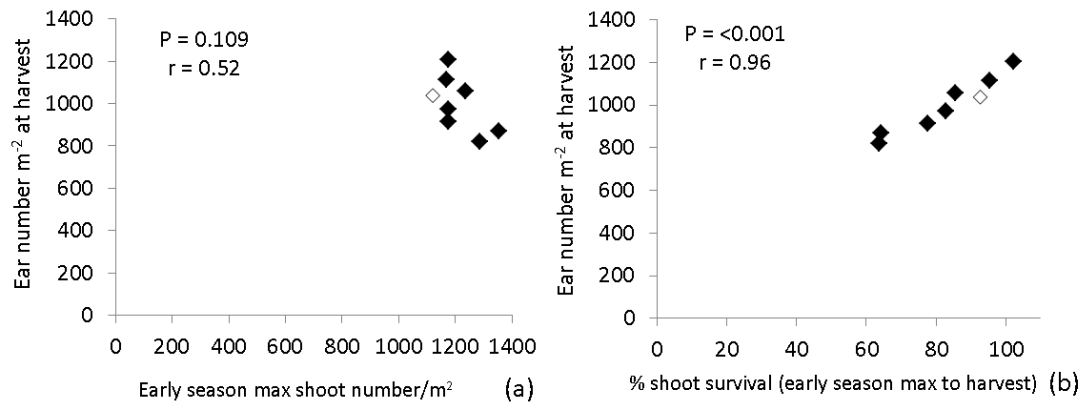


Figure 2.16. Plots of early season maximum shoot number m^{-2} versus ear number m^{-2} at harvest (a) and % shoot survival (early season maximum to harvest) versus ear number m^{-2} at harvest (b). Results of correlation analysis are given. Data excludes CW 2013. Unfilled marker is the CK 2011 data point where the harvest ear number m^{-2} value was estimated from the previous growth analysis sample date.

There was a statistically significant correlation between total biomass per shoot at GS31 and % shoot survival from the identified early season maximum to mid-season minimum (Table 2.4, $P = 0.013$, $r = 0.619$). When this biomass was broken down into component plant fractions, the strongest correlation was between leaf biomass per shoot and % shoot survival ($P = 0.007$, $r = 0.83$). There was also a similarly strong correlation with the % of light intercepted per shoot at GS31, but not with shoot number m^{-2} at GS31. There were weak but non-significant ($0.10 > P > 0.05$) correlations between green area per shoot and shoot N content at GS31 and % shoot survival. A negative correlation between shoot number m^{-2} at the early season maximum and % shoot survival ($P = 0.014$, $r = -0.78$) illustrates that where initial shoot production was high there was less % shoot survival. Plant number m^{-2} which could be used as a proxy of the number of main stems at the time of early season maximum shoot number had no relationship with % shoot survival. The duration of stem extension (from GS31 to anthesis) was significantly correlated with % shoot survival ($P = 0.017$, $r = -0.76$); the correlation was negative where the longer the duration of stem extension the lower the % shoot survival. The duration of the shoot death period itself was not significantly correlated with % shoot survival ($P = 0.162$,

$r = 0.43$); the same was true for the duration of the period from emergence to GS31. However there was a strong correlation between the rate of biomass accumulation from emergence to GS31 and % shoot survival ($P = 0.003$, $r = 0.88$). There was no significant correlation between the rate of biomass growth during stem extension (GS31 to anthesis) and % shoot survival.

Table 2.4. Correlation analysis of the relationship between % shoot survival (from the early season maximum shoot number to the mid-season minimum shoot number) and a range of growth variables at GS31, early season maximum shoot number, pre-GS31 and post-GS31. CW 2013 data excluded.

	<i>P</i>	<i>r</i>
Total biomass per shoot at GS31 (g shoot ⁻¹)	0.013	0.79
Stem and sheath biomass per shoot at GS31 (g shoot ⁻¹)	0.044	0.66
Leaf biomass per shoot at GS31 (g shoot ⁻¹)	0.007	0.83
% light interception per shoot at GS31	0.003	0.87
Shoot number m ⁻² at GS31	0.169	-0.41
Total green area at GS31 (cm ² shoot ⁻¹)	0.091	0.55
Stem and sheath green area at GS31 (cm ² shoot ⁻¹)	0.371	ns
Leaf green area at GS31 (cm ² shoot ⁻¹)	0.080	0.57
Total N at GS31 (g shoot ⁻¹)	0.073	0.59
Stem and sheath N at GS31 (g shoot ⁻¹)	0.076	0.58
Lamina N at GS31 (g shoot ⁻¹)	0.129	0.48
Shoot number m ⁻² at early season maximum shoot number	0.014	-0.78
Plant number m ⁻² (also number of main stems m ⁻²)	0.603	ns
Duration GS31 to anthesis (days)	0.017	-0.76
Duration of shoot death period (early season max to mid-season min, days)	0.162	-0.43
Duration emergence to GS31 (days)	0.402	ns
Rate of biomass accumulation emergence to GS31 (t ha ⁻¹ day ⁻¹)	0.003	0.88
Intercepted PAR emergence to GS31 (MJ m ⁻²)	0.613	ns
Rate of biomass accumulation GS31 to anthesis (t ha ⁻¹ day ⁻¹)	0.172	0.41
Intercepted PAR GS31 to anthesis (MJ m ⁻²)	0.878	ns

There was no relationship between mid-season minimum shoot number m⁻² (before re-tillering) and the harvest ear number m⁻² (Figure 2.17). Mean harvest values were

higher than mid-season minimum values at all site/seasons except CW 2013 where potentially fertile shoot number decreased in the run up to harvest. There was a significant site \times season \times time interaction effect on shoot number/m² ($P = 0.014$, L.S.D. 161.5) showing that harvest ear number m⁻² was significantly higher than mid-season minimum shoot number m⁻² at two sites (WX 2011 and CK 2012) (Figure 2.17).

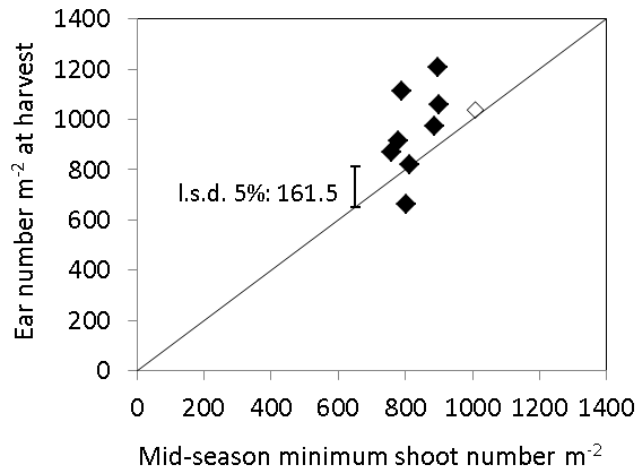


Figure 2.17. Scatter plot of mid-season minimum shoot number m⁻² (x-axis) plotted against ear number m⁻² at harvest with a 1:1 line. Values are means of three replicates. Unfilled marker is the CK 2011 data point where the harvest ear number/m² value was estimated from the previous growth analysis sample date. L.S.D. 5% for the site \times season \times timing interaction effect obtained from ANOVA is also shown.

Correlation analysis (excluding CW 2013), showed that light interception at the mid-season minimum time-point was not correlated with the % shoot increase from the mid-season minimum to harvest ($P = 0.541$). The same was true for values of accumulated rainfall for one week ($P = 0.360$) and two weeks ($P = 0.525$) prior to the mid-season minimum.

2.3.7 Realisation of high yield potential

Linear regression analysis has shown that there was no relationship between grain number m^{-2} and MGW for the 9 site/seasons ($P = 0.554$). Strong relationships were found between grain number m^{-2} and harvest ear biomass, harvest total biomass, and accumulated ear biomass post-anthesis (Table 2.5). The relationship between grain number m^{-2} and accumulated total biomass post-anthesis, while significant, was not as strong ($P = 0.024$, $R^2 = 0.47$). There was no significant relationship between grain number m^{-2} and stem biomass decline from anthesis to physiological maturity (GS55 to GS87). There was no significant relationship between grain number m^{-2} and PAR_{int} post-anthesis and RUE post-anthesis.

Table 2.5. Linear regression analysis of grain number m^{-2} versus measures of growth post-anthesis, intercepted photosynthetically active radiation (PAR_{int}) post-anthesis and radiation use efficiency (RUE) post-anthesis for all 9 site/seasons of data

	<i>P</i>	<i>R</i> ²
Harvest ear biomass (t ha^{-1})	<i><0.001</i>	<i>0.84</i>
Harvest total biomass (t ha^{-1})	<i><0.001</i>	<i>0.92</i>
Accumulated ear biomass post-anthesis (t ha^{-1})	<i>0.002</i>	<i>0.74</i>
Accumulated total biomass post-anthesis (t ha^{-1})	<i>0.024</i>	<i>0.47</i>
Stem biomass decline GS55 to GS87 (t ha^{-1})	<i>0.685</i>	<i>ns</i>
PAR_{int} post-anthesis (MJ m^{-2})	<i>0.117</i>	<i>0.21</i>
RUE post-anthesis (g MJ^{-1})	<i>0.375</i>	<i>ns</i>

2.4 Discussion

Across 9 site/seasons, the grand means achieved of 8.52 t ha⁻¹ for yield, 18,419 for grain number m⁻² and 46.41 mg for MGW were close to those of similar work in the UK on winter barley where grand means of 8.8 t ha⁻¹ for yield, 18,600 for grain number m⁻² and 46 mg for MGW were achieved across 18 site/seasons (Blake et al., 2006). Given that spring barley in any given environment can yield about 20% less than winter barley, the yield and yield component values achieved in the current study illustrate the high yield potential of barley in the Irish climate. Mean values of 5.67 t ha⁻¹ for yield, 12,151 for grain number m⁻² and 47.21 mg for MGW achieved for a similar seed rate across 4 varieties and 6 site/seasons in a spring barley study in the UK confirm this (Wade and Froment, 2003).

Significantly higher yields in 2011 than in 2012 and 2013 were accompanied by significantly higher grain numbers. A cooler than average mid- to late-season resulted in a longer duration of grain filling and rapid ear growth in 2011 than in 2012 and 2013. Slightly above average solar radiation post-anthesis, ensured that the mean growth rate for the rapid total biomass and rapid ear growth periods in 2011 were high in comparison to subsequent seasons. As a result, the high potential grain numbers established early in the season translated into high yields.

A significantly lower MGW in 2012 than in 2011 and 2013 may be partly attributed to low solar radiation levels. Despite favorable (cool) temperature curves producing the longest mean duration of rapid total biomass growth in 2012, the mean growth rate for the same period was the lowest of the three seasons. Ear blight can result in shriveled grain of a reduced size in small grain cereals (Kazan et al., 2012; McMullen et al., 1997; Osborne and Stein, 2007; Parry et al., 1995) and given its prevalence across the three sites in that season may also have contributed to the lower MGW in 2012.

In comparison to all other site/seasons CW 2013 had a particularly low yield and grain number m⁻². Here, the crop which was sown at the traditional timing for the area (mid-March), was subject to an unusually cool and damp spell immediately post-sowing. Emergence is heavily dependent on temperature (Newton et al., 2011)

and the low temperatures coupled with a cloddy seedbed (rain prevented rolling of the soil post-sowing) reduced seedling emergence. This resulted in the lowest plant population (183 plants m⁻²) and significantly lower ear number m⁻² at harvest (664 ears m⁻²) than all other site/seasons. The crop did not recover from the poor start and canopy closure did not occur. Crops at the other two sites in 2013 were not affected similarly as they were sown in early April into more favourable conditions.

Grain number m⁻² was strongly related to yield across sites and seasons, supporting previous findings in the literature across a range of environments (Abeledo et al., 2003; Bingham et al., 2007a; Blake et al., 2006; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013). This is evidence supporting the hypothesis that grain number m⁻² determined yield in the high yield potential Irish climate.

Various source:sink manipulation experiments across a range of environments on barley (Arisnabarreta and Miralles, 2008b; Grashoff and d'Antuono, 1997; Habgood and Uddin, 1983; Willey and Holliday, 1971) wheat (Abbate et al., 1997; Fischer, 1985) and triticale (Estrada-Campuzano et al., 2008) highlight the importance of the stem extension period for grain number determination through its influence on grain number survival. These authors highlight the influence of grains per ear rather than ear number m⁻² on grain number formation during this period and other theoretical discussions on yield improvement in wheat concur with this (Miralles and Slafer, 2007; Reynolds et al., 2000). However, in the current study ear number m⁻² at harvest largely determined grain number m⁻² with ear number m⁻² itself largely determined by % survival from an early season maximum through to harvest. Grain number per ear accounted for little of the variation thus supporting the hypothesis that grain number m⁻² is most readily influenced by ear number m⁻². Linear regression analysis of data obtained from a recent UK study on spring barley of 4 varieties and 5 seed rates across 6 site/seasons (Wade and Froment, 2003) also shows that ear number largely determined grain number ($P < 0.001$, $R^2 = 0.56$) while grain number per ear accounted for little of the variation ($P < 0.001$ $R^2 = 0.09$). Given that grain number per ear is more conserved in barley than in wheat (Wade and Froment, 2003) particularly in two-row barleys such as cv. Quench which are limited by the number of grains per ear they can bear (Arisnabarreta and Miralles, 2008a) the divergence

from the literature on wheat is unsurprising. However the contradiction of the present study from the barley literature quoted above is surprising given that the literature quoted used almost exclusively 2-row type barleys similar in type to Quench (Arisnabarreta and Miralles (2008b) used both 2-row and 6-row types). Given that the barley experimentation in the literature quoted was carried out in Argentina, Netherlands and the UK differences in temperature, rainfall, solar radiation and yield potential may have resulted in an alternative determination of grain number m^{-2} . Also, where data was presented in the literature and comparable to the present study (Grashoff and d'Antuono, 1997; Willey and Holliday, 1971), mean harvest ear number m^{-2} values for control treatments (approx. 600 – 800) were lower than the current study mean of 961. While a degree of trade-off exists between ear number m^{-2} and grain number per ear at high ear numbers (Wade and Froment, 2003; Willey and Holliday, 1971), it is also possible that a low ear number m^{-2} may result in a more plastic grain number per ear (Baethgen et al., 1995).

Given that harvest ear number m^{-2} accounted for most of the variation in grain number m^{-2} the mechanisms responsible for its determination are of interest. The tillering pattern of barley can involve maximum production around the beginning of stem extension (GS31) followed by death during stem extension then stabilisation from anthesis through to harvest (del Moral et al., 1984; Gallagher et al., 1975; Slafer et al., 2009). While early season shoot number maxima followed by periods of shoot mortality were identified at all site/seasons, the maxima occurred at or around GS31 at only 6 site/seasons. At the other site/seasons this maximum occurred later, particularly so at CW 2013 where canopy closure did not occur due to poor crop establishment and as such maximum shoot number was not reached until after anthesis. For this reason CW 2013 data points were excluded from various regression analyses investigating tillering dynamics. With CW 2013 excluded, the shoot mortality period began pre-anthesis at all sites/seasons but was not completed until after anthesis in five site/seasons. It is clear that the period of tiller production in barley is quite plastic (Simmons et al., 1982; Willey and Holliday, 1971). It is also clear that the period of tiller senescence is similarly plastic and is not always completed before anthesis; this is in agreement with some published literature on wheat and barley (Lauer and Simmons, 1989; Sparkes et al., 2006). While the

contribution of late season tillering to final ear number m^{-2} and yield is thought to be negligible (Kirby, 1967; Thorne and Wood, 1988), the current study has shown that in two out of nine site/seasons, post-anthesis re-tillering did contribute significantly to harvest ear number m^{-2} and hence yield.

In a spring-sown barley crop (cv. Proctor) of standard seed rate in the UK a maximum of 1500 shoots m^{-2} (including main stems and tillers) was produced (Gallagher et al., 1976). This is somewhat comparable to the mean early-season maximum of 1212 shoots m^{-2} identified in the current study. At site/seasons in the current study where early season maximum shoot number was high there was lower shoot survival, however, early season maximum shoot number m^{-2} had no relationship with ear number m^{-2} (and hence yield) at harvest. Variation in ear number m^{-2} at harvest was almost completely explained by variation in shoot survival from the early season maximum through to harvest. Tiller mortality can vary with cultivar and environment (Kirby and Riggs, 1978; Simmons et al., 1982; Thorne, 1962) and survival rates of 68% to 37% (from maximum shoot number to harvest shoot number) have been recorded in field studies of several varieties and types of barley in contrasting Mediterranean environments (del Moral and del Moral, 1995). With CW 2013 excluded (because it did not reach maximum shoot number until post-anthesis), a mean shoot survival of 71% was achieved across the remaining eight site/seasons. This is at the higher end of the range set out by (del Moral and del Moral, 1995). However as stated previously, this was a net effect of early-mid season shoot death and post-anthesis re-tillering. As such the mechanisms controlling both the shoot death from the early season maxima to the mid-season minima identified and the subsequent post-anthesis re-tillering warranted further investigation.

Measures of shoot dry weight and size at GS 31, in particular the leaf portion of the shoots, had a greater influence on the proportion of shoots surviving from the early season maxima to the mid-season minima than measures of growth during stem extension did. However where the duration of stem extension was longer there was lower shoot survival. Crops that intercepted more light per shoot at GS 31 had a greater shoot survival and this was not simply due to thinner crops having more assimilate available per shoot during stem extension as there was no relationship

between shoot number m^{-2} at GS 31 and shoot survival. A strong relationship between the rate of biomass accumulation from emergence to GS 31 indicates that factors contributing to individual shoot size, mass and ability to independently produce assimilates at GS 31 will most likely influence shoot survival. The lack of relationship between plant population and shoot survival indicates that shoot survival was not proportionate to the number of main stems present and that non-main stem tillers did reach a size and green area sufficient to independently produce the photoassimilates they require throughout stem extension (del Moral et al., 1984; Kirby, 1977).

A flush of late season tillering in response to a rainfall event is possible (Kirby, 1967) particularly following a period of drought (Jamieson et al., 1995). It was difficult to test this hypothesis in the current study due to the absence of soil moisture data however accumulated rainfall in the week and two weeks prior to re-tillering events did not explain the variation in % shoot increase from mid-season minimum to harvest, neither did any potential effects of increased solar radiation availability low in the canopy following a possible thinning effect of shoot death. If post-anthesis re-tillering did not contribute to harvest ear number m^{-2} then a 1:1 relationship would be expected between the mid-season minimum shoot number m^{-2} (before re-tillering) and the harvest ear number m^{-2} . This was not the case and harvest ear number was significantly higher than mid-season minimum shoot number m^{-2} at WX 2011 and CK 2012. As such, post-anthesis re-tillering must be considered as a real contributor to final harvest ear number and hence yield in at least two of the nine site/seasons.

Strong relationships between grain number m^{-2} and harvest ear biomass, harvest total biomass and accumulated ear biomass post-anthesis are unsurprising given the strong relationship between grain number m^{-2} and yield – high grain number m^{-2} crops realised their high yield potential. The mechanisms responsible for this are unclear with the variation in grain number m^{-2} across site/seasons not accounting for the variation in either PAR_{int} post-anthesis, RUE post-anthesis or the decline in stem biomass from GS55 to GS87 (notwithstanding the fact that the decline in stem biomass from GS55 to GS87 could be a consequence of respiration as well as

remobilization). It would appear that the relative contribution of each of the three variables differs across sites and seasons.

RUE declined or ‘leveled-off’ late season in two out of nine site/seasons (WX 2011 and CK 2011). At these two site/seasons green tissue continued to intercept radiation towards the end of grain filling but crops accumulated less dry matter per unit of intercepted radiation than they did earlier. Stem biomass decline began around the same time that RUE leveled-off and grain growth did not complete until approximately 2-3 weeks later. Grain growth may have been fuelled by means other than direct photosynthesis, such as stored stem carbohydrate reserves (Beed et al., 2007; Bingham and Topp, 2009; Fabian et al., 2011; Foulkes et al., 2007; Serrago et al., 2013; Yoshida, 1972). Total biomass growth began to slow down before total ear growth at all site/seasons and this could be interpreted as further evidence of utilisation of stored carbohydrate reserves for grain filling but does not explain the decline in RUE at WX 2011 and CK 2011. An increase in respiration relative to photosynthetic activity with senescence (Bingham et al., 2007a) or a decline in photosynthetic efficiency with leaf age (Biscoe et al., 1975c) may be responsible. There may also exist a feedback control mechanism whereby photosynthetic activity is down regulated due to a limited sink demand (Bingham et al., 2007a) thereby reducing RUE below its potential (Newton et al., 2011). However, WX 2011 and CK 2011 both had a significantly higher grain number m^{-2} than the other site in that season CW 2011 where RUE did not ‘level-off’ post-anthesis. In fact WX 2011 and CK 2011 had the highest and second highest grain numbers m^{-2} of all nine site/seasons. It is unlikely that if a limited sink demand was responsible for the down regulation of photosynthesis that it was due to a limited grain number m^{-2} . The limited sink demand may however have occurred in the form of a limited grain storage capacity, where the ability of the grain to accumulate dry matter was restricted (Bingham et al., 2007b) – MGW’s at WX 2011 and CK 2011 were lower than CW 2011 (non-significant at WX 2011). Either way a surplus of assimilate for grain filling is implied at these site/seasons.

2.5 Conclusion

In conclusion, Ireland has a high yield potential for spring barley. The hypothesis that grain number of spring sown-barley determines yield in the high yield potential Irish climate can be accepted. Survival of potential grain sites had a strong influence on harvest grain number determination but in contrast to the literature it was the survival of ears rather than grains/ear that was of greater importance. As such the hypothesis that grain number of a two-row spring barley variety in Ireland is most readily influenced by ear number can be accepted. The period over which ear number was determined was more flexible than the literature suggested where ear number was largely determined by ear survival from an early season maximum through to harvest including significant post-anthesis re-tillering in two out of nine site/seasons. Shoot size and weight at GS31 had the largest influence on shoot survival indicating that crop condition at GS31 and hence growth and development pre-GS31 may be more important for shoot survival than growth and development during the stem extension period. High shoot numbers were required to achieve a high yield potential crop but there was no relationship between early season maximum shoot number and harvest ear number. Achieving high shoot numbers of adequate size and weight at GS31 may be an appropriate target for establishing a high yield potential crop.

Chapter 3 **Grain number response to post-anthesis reductions in assimilation capacity**

3.1 Introduction

Grain number is highly correlated with yield in barley crops grown in a range of environments (Abeledo et al., 2003; Baethgen et al., 1995; Bingham et al., 2007a; Blake et al., 2006; del Moral et al., 2003; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013) while grain weight tends to be poorly correlated (Abeledo et al., 2003; Baethgen et al., 1995; Blake et al., 2006; Bulman et al., 1993; Gallagher et al., 1975; Sadras and Slafer, 2012; Wade and Froment, 2003). The previous chapter provides evidence that this is also the case in the high yielding environment of Ireland. Further, when yield component data from this thesis were pooled with those from other experimental work on spring barley in the UK and Ireland (Bingham et al., 2010; Conry, 1995, 1998; Wade and Froment, 2003; Willey and Holliday, 1971), the coefficient of variation (cv) for grain weight was 10.5 compared to 27.6 for grain number (based on 506 observations comprising mean values from both hand threshed and combine threshed sources across several sites and seasons and a range of agronomic treatments including variety, seed rate, sowing date, nitrogen rate, nitrogen timing, fungicide rate and fungicide timing). This translated into a range of variation in MGW of 93% (from minimum to maximum value) compared with a range of 831% for grain number. The analysis implies that grain weight of barley is the most conserved of the two primary yield components across a range of sites, seasons, yields, and agronomic factors, consistent with reports for other species (Bradshaw, 1965; Harper, 1977; Sadras, 2007; Sadras and Slafer, 2012).

Sadras and Slafer (2012) suggest that there is a hierarchy of plasticity amongst yield components of small grained cereals from tiller number (the most plastic and least conserved) to seed size (the least plastic and most conserved). They argued that the hierarchy reflects the differential costs and contribution to fitness of the yield components, the stabilizing effects of natural and artificial (plant breeding) selection for seed size, and changes in resource availability during the life of the crop. Investment of resources in the production of seed within a relatively narrow range of

sizes could have benefits for the plants' reproductive fitness as larger seed with larger embryos and storage reserves have a greater chance of producing seedlings that establish successfully, are able to compete with neighbouring plants and tolerate damage from herbivores (Sadras, 2007).

At present the physiological mechanisms that underlie this apparent conservation of MGW are poorly understood. In particular it is unclear how assimilate availability for grain filling can be maintained in the face of a highly variable grain number in a way that ensures all grains fill adequately when grain number is largely determined *before* grain filling occurs. Evidence suggests that for barley and other grain crops, photosynthetic capacity at anthesis and beyond is not limiting yield (Bingham et al., 2007a; Dreccer et al., 1997; Richards, 2000; Serrago et al., 2013; Slafer and Savin, 1994) and that grain growth may also be fuelled by means other than direct photosynthesis, such as the remobilisation of stored stem carbohydrate reserves deposited pre- and early-post anthesis (Austin et al., 1977; Austin et al., 1980; Beed et al., 2007; Bingham and Topp, 2009; Fabian et al., 2011; Foulkes et al., 2007; Gallagher et al., 1975; Serrago et al., 2013; Yoshida, 1972). The contribution of storage reserves to grain filling can vary with environment, season and sink size (Gallagher et al., 1975) and stem reserves may only be utilised if the photosynthetic capacity of the crop is reduced post-anthesis (Grashoff and d'Antuono, 1997; Nosberger and Thorne, 1965; Serrago et al., 2013). In such cases, stored carbohydrate reserves may act as a buffer to maintain a steady rate of grain filling (Ehdaie et al., 2006) and may simply remain underutilised if no deficit exists (Gallagher et al., 1975). In addition, there is evidence that post-anthesis RUE might be regulated in response to variations in sink demand from the grain (Bingham et al., 2007a; Calderini et al., 1997; Miralles and Slafer, 2006; Reynolds et al., 2005). Thus MGW might be conserved through adjustments in utilisation of storage reserves and photosynthetic rate according to the assimilate demand of the grain enabling a relatively constant MGW to be maintained irrespective of the number of grain set (Gallagher et al., 1975).

It has also been suggested that grain numbers themselves might be adjusted to match the potential of the crop to provide assimilate for grain filling. This is implicit in the

model of yield determination for wheat proposed by Sinclair and Jamieson (2006). They argued that rather than grain number m^{-2} determining yield, yield is simply a consequence of resource accumulation and use by the crop and that grain number m^{-2} is adjusted to match the resource defined yield level. If, as is widely accepted, grain number is determined pre-anthesis (Arisnabarreta and Miralles, 2008a, b; Fischer, 1985; Ghiglione et al., 2008; Miralles et al., 2000; Sadras and Denison, 2009; Sinclair and Jamieson, 2006) then this would require some means of ‘predicting’ potential post-anthesis assimilate supply before flowering. This might involve resource-based mechanisms in which floret and tiller survival are regulated by assimilate availability and organ or crop growth rate during late stem extension (Gallagher et al., 1976; Hay and Kirby, 1991; Prystupa et al., 2004; Sadras and Slafer, 2012; Slafer et al., 2009). High growth rates and high levels of assimilate availability would be indicative of crops with a large photosynthetic capacity and potential stem storage reserve and thus crops able to support large grain numbers during grain filling. However, there is increasing evidence that non-resource factors such as photoperiod and the spectral composition of light can influence grain number formation and provide environmental and developmental cues to allow plants to predict the future availability of resources (Davis and Simmons, 1994; Ghiglione et al., 2008; Gonzalez et al., 2003, 2005; Sadras and Slafer, 2012; Skinner and Simmons, 1993; Sparkes et al., 2006; Ugarte et al., 2010).

The post-anthesis abortion of grains is a third potential mechanism that could contribute to the conservation of grain weight as this would allow grain numbers to be fine-tuned to the amount of assimilate available during grain filling. Gallagher et al. (1975) proposed the hypothesis, but subsequently dismissed it because the evidence at the time suggested that grain number per unit area was firmly fixed by anthesis. More recent evidence has shown that the period of grain number determination is quite plastic and that a mechanism for post-anthesis grain number adjustment may exist. Adjustments in grain numbers of barley and other small grain crops have been observed following a range of post-anthesis treatments including shading, artificial temperature modification, drought and crop-thinning (Boyer and McLaughlin, 2007; Boyer and Westgate, 2004; Estrada-Campuzano et al., 2008; Grashoff and dAntuono, 1997; Habgood and Uddin, 1983; Nicolas et al., 1985;

Zinselmeier et al., 1999). These reductions in grain number were due to adjustments in ear number per unit area and/or grain number per ear. Moreover in maize, embryo abortion during drought could be prevented by the exogenous supply of sugars suggesting that abortion was a response to a restricted availability of carbon assimilates (Boyer and McLaughlin, 2007; Boyer and Westgate, 2004). These observations suggest that there is a mechanism for adjusting the number of grains according to the post-anthesis assimilation capacity. If the proposed down-regulation of grain number is to ensure that each grain has a good chance of filling adequately and to reach a size that would maximise the chances of successful seedling establishment, such an adjustment would be expected to occur in less developed distal positions of the ear where potential grain size and ability to compete for resources is the smallest (Gallagher et al., 1976; Kirby, 1977; Nicolas et al., 1985) or on later produced tillers given that tiller survival is generally greater for earlier produced tillers (Cannell, 1969; Davidson and Chevalier, 1990; Gallagher et al., 1976; Kirby and Riggs, 1978). In wheat, growth of grains in distal florets of spikelets was more sensitive to shading treatments than those nearer the base of the spikelet, but different spikelets were affected similarly (Bremner and Rawson, 1978). If grain numbers are adjusted post-anthesis in order to regulate grain size within a relatively narrow range, then agronomic or breeding attempts to improve yield by modifying pre-anthesis crop growth to increase grain numbers may be unsuccessful unless they simultaneously ensure a greater post-anthesis assimilate supply for grain filling. The mechanisms at play are unclear for barley and have received relatively little attention in high yielding environments where post-anthesis drought is rare, such as Ireland. The main objective of experiments in this chapter was, therefore, to investigate whether grain number in spring barley is adjusted post-anthesis in response to large scale modifications in post-anthesis photosynthetic activity.

Before treatment effects on grain number and MGW can be investigated it is important to establish the reliability of techniques for estimating each in field plots. After fertilization of the ovule, the embryo and endosperm tissues develop. The presence of one or both of these structures, however small, indicates that the grain has acted as a sink for post-anthesis assimilate and hence justifies its inclusion as a grain for the purposes of scientific investigations of source-sink relations. A combine

harvester uses a series of adjustable threshing mechanisms, sieves and fans to get a 'clean' sample of marketable and usable grains. In doing so, smaller and lighter grains similar in physical properties (especially weight) to that of the chaff, may be lost from the harvested sample. Such a systematic inaccuracy could result in an overestimation of MGW and underestimation of grain number (Bloom, 1985; Gallagher et al., 1975). For example, if assimilate for grain filling becomes limiting in crops of large grain number resulting in small under-filled grains at harvest, these grains may be lost from the sample resulting in a skewed grain weight distribution, and as such contribute to an apparent conservation of MGW. In this instance, hand harvesting and hand threshing crop samples to obtain yield component data may be a more appropriate method. A second objective of this chapter was, therefore, to compare grain weight distributions in samples of viable grain from combine harvested and hand harvested plots to quantify the impact of potential grain losses from the combine on estimates of MGW.

The specific hypotheses tested were:

- The loss of small grains from combine harvesters contributes to the apparent conservation of MGW
- Reduction in assimilation capacity post-anthesis reduces grain number in Irish-grown spring barley

3.2 Materials and Methods

3.2.1 Harvesting method and mean grain weight

Grain samples from experiments reported in Chapter 2 and Chapter 4 were used to investigate the effects of harvest and threshing method on estimates of mean grain weight (MGW). Full details of experimental treatments and crop husbandry are given in the relevant chapters; only a brief summary is provided here.

3.2.1.1 Comparison of harvest and threshing method across sites, seasons and seed rate treatments

MGW of combine- and hand-threshed samples obtained from the nine site/seasons of experiments discussed in Chapter 2 were analysed for effects of harvesting method and site x season x harvesting method interactions.

Experimental design at each experimental site/season consisted of three plots for destructive sampling (three replicates) alternated with three plots for combine harvested yields (three replicates) within one bank of six plots. Plots were 4 m wide and ranged in length from 21 m to 24 m. The variety was cv. Quench and plots were managed for high yield potential. At harvest, combine threshing and separation apparatus were set up with the objective of achieving a clean sample while losing as little grain as possible. On the same day as plot harvesters sampled the combine harvested plots, a quadrat sample size of 6 x 1 m adjacent row lengths of crop was removed from the plots designated for destructive sampling and air dried prior to processing. Ears in a 20% sub-sample were dried at 70 °C for 48 hours (or to a constant mass) and then hand-threshed between two pieces of foam board and sieved over a mechanically operated 1 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate into chaff and grain portions.

MGW data were analysed using a general ANOVA treatment structure with GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK). The model was as follows: season*site*harvest method + site/block. Block was nested in site to account

for the fact that blocks at one site were distinct from blocks at another site. Statistical power is weakened by the nesting therefore such an analysis is not strictly valid for investigating cause and effect but for observational interpretation only (J. Grant, personal communication, 2014).

In 2013, field experiments were established at Carlow (CW) and Kilkenny (KK), with spring barley cv. Quench sown at seed rates of 40, 80, 160, 320, 640 and 1280 seeds m^{-2} . These experiments were aimed at investigating the relationship between grain number and MGW and are described in more detail in Chapter 4, section 4.2.2. In this chapter, the effects of harvesting method on estimates of MGW were determined using samples from the seed rate treatments which varied in grain weight. At each site the experiments were laid out in one bank of plots with four fully randomised blocks of six seed rate treatments each. Plots were 2 m wide and 24 m long with half the length of the plot designated for combine harvesting and the other half for destructive sampling. Plots were managed for high yield potential. Combine harvesting, pre-harvest destructive sampling, sample storage and hand-threshing were as described in Chapter 2. MGW was calculated for each sample using an automated grain counter (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate 25 g grain sample. After counting, grain weight was determined to the nearest 0.1 mg. Mean grain weight from the two experiments were subject to ANOVA (two-way factorial in randomized blocks) using GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK) with harvesting method (hand threshed or combine threshed) and seed rate as the two factors of interest.

3.2.1.2 Grain weight distribution

Grain samples from two experimental sites discussed in Chapter 2 were used to determine the grain weight distribution for hand-threshed and combine-harvested plots. Samples were from 2011 field experiments at Carlow (CW) and Wexford (WX).

One hundred grains per replicate were weighed individually giving three hundred grains per harvest method. Samples were poured onto a tray, mixed well (samples were not shaken) and spread across the tray. Grains were then selected and weighed individually to 0.1 mg working from one end of the tray to the other until one hundred grains were weighed. The histogram function in Microsoft Excel (Microsoft Office 2010, Microsoft Corporation, Washington, USA) was used and weight classes were set at an interval of 2.5 mg. The number of grains in each class was then expressed as a % of the total number of grains weighed.

3.2.1.3 Vital staining

To test whether hand-threshed grain samples contained some non-grain material imbibed seeds of defined size class were stained with 2,3,5 Triphenyltetrazolium Chloride (TTC) (Sigma-Aldrich Co.).

Air dried hand-harvested ear subsamples (20% of the pre-harvest 6 x 1 m destructively sampled quadrat) were retained from the Kilkenny (KK) seed rate experiment described in section 3.2.1.1. Ear counts were carried out before samples were hand-threshed and sieved over a 1 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate chaff from grain as described in section 3.2.1.2. Mean grain weight (MGW) was calculated for each plot using an automated grain counter (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate 25 g grain sample. Grain number m^{-2} was then calculated using MGW and plot yield data, and grain number per ear was calculated using grain and ear number data.

Grain portions were then sieved over a 1.75 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate suspected non-grains from the grain sample. All suspect grains fell through the 1.75 mm sieve. The resultant 1 mm < 1.75 mm size class consisted of approx. 30-60 suspected non-grains and possible grains.

Material from each plot sample was imbibed in distilled water for 18h at 20°C on individual petri dishes. A large reference grain (from the > 1.75 mm size class) from

the same sample with an obvious endosperm and embryo was placed in the center of each petri dish to validate that the staining had worked. Excess water was drained following the soaking period and grains were then cut longitudinally through the embryo with a scalpel and $\frac{3}{4}$ of the way through the endosperm. Where embryos and endosperms were indistinguishable on potential non-grains the cut was made through the crease region at the expected location of these two structures. A 1.0% v/v TTC solution was prepared in distilled water and 25 ml added to each Petri dish. Aluminium foil was placed around the petri dishes to exclude light and grains were incubated in this solution for 3 hours at 30 °C. Following this, the two halves of the seed were spread out and the external surface of the embryo was observed with the naked eye. Viable embryo tissue was stained red and was distinct from the non-stained white endosperm. Grains (with both an embryo and endosperm) and non-grains (everything else) were then separated and counted.

Material was oven dried at 70 °C for 48 hours (or to a constant mass) and weighed. The % of non-grains in the entire grain sample, number of non-grains m^{-2} , and number of non-grains per ear were then estimated.

Data obtained were statistically analysed for seed rate effects using ANOVA as above. Ear number m^{-2} data were plotted against number of non-grains m^{-2} data and fitted with an exponential curve regression using Microsoft Excel (Microsoft Office 2010, Microsoft Corporation, Washington, USA).

3.2.1.4 Adjusting hand-threshed yield data to account for the presence of non-grains

Given that vital staining identified non-grains in hand-threshed grain samples a method was derived to retrospectively adjust hand-threshed yield and yield component data to account for the presence of non-grains in samples. Due to the strong influence that ear number m^{-2} had on the number of non-grains m^{-2} it was decided that a unique reduction factor would be calculated based on the ear number of the plot/treatment/crop in question. The calculated ear number m^{-2} for any particular sample was entered into the equation of the line from the regression of ear

number m^{-2} and non-grains m^{-2} from the staining experiment (Figure 3.5). The number of non-grains m^{-2} for that particular sample was then estimated and subtracted from the overestimated grain number m^{-2} figure. Yield was adjusted by working out the number of non-grains m^{-2} as above and multiplying by the mean non-grain dry weight of 2.9 mg (calculated from the staining experiment). This weight of non-grains was converted to t ha^{-1} , adjusted to 85% dry matter and subtracted from the yield. The corrected grain number m^{-2} figure was then divided into the corrected yield figure to get a corrected MGW. Grain number per ear was also corrected from the new grain number m^{-2} figure and existing ear number m^{-2} values.

3.2.2 Shading to reduce photosynthetic assimilation capacity post-anthesis

3.2.2.1 Experimental design, treatments and husbandry

Field experiments involving shading post-anthesis were employed to test the hypothesis that a reduction in assimilation capacity post-anthesis reduces grain number in Irish-grown spring barley. Shading treatments were applied to crops of a high yield potential two-row spring barley variety (*Hordeum vulgare* L., cv. Quench) at Oakpark, Co Carlow (CW) in 2011 and 2012. Site and crop management details were as per standard farm practice and are described in Chapter 2, section 2.2.1. A seed rate of 330 seeds m^{-2} was used in both seasons.

Plot size in 2011 was 2 m x 3 m with 3 m discard plots between shaded areas and control plots to avoid overshadowing. Shading treatments were applied to entire plots in 2011. Plot size in 2012 was 4 m x 24 m and shades were erected over sub-plots of 2 m x 3 m. Treatment areas in both seasons were further sub-divided into two sampling areas – one for destructive sampling of ears for grain growth assessment during the treatment period and one for final grain number and biomass determination at harvest. The position of these sampling areas alternated with each replicate.

The shading material used was an open weave polystyrene shade-netting (Tildenet Ltd., Bristol, UK). Shades were erected on a frame of fencing posts and rope at a height of 1.1 m above ground level (Figure 3.1). Anthesis was judged to occur when half of the ear had emerged on half of all shoots which corresponded to GS 55 on the Zadoks scale. A single ‘late’ shading treatment was applied in 2011 from 14 days after Zadock’s GS 55 (Tottman, 1987) until physiological maturity and compared to a control in a one-way randomized block design with six replicates. The ‘late’ shading was repeated in 2012 alongside an additional ‘early’ shading treatment applied at GS 55 for a period of 14 days. A one-way randomized block design was used in 2012 with four replicates.



Figure 3.1. A ‘late’ shading treatment area at CW in 2011.

3.2.2.2 In-field measurements

Plant population counts were carried out by counting the number of plants both sides of a 0.5 m marker at five locations per plot and converted to area based measurements using the row width. A pyranometer (SPLite2, Kipp & Zonen B. V., Delft, Netherlands) and a relative humidity/temperature probe (MP100A, Rotronic Instruments (UK) Ltd., Crawley, UK) connected to a data logger (CR1000, Campbell

Scientific Ltd., Loughborough, UK) and installed in 'late' shaded and unshaded treatment areas in 2011. Environments were monitored for solar radiation, relative humidity and temperature differences throughout the treatment period. In all seasons, soil was sampled to 30 cm using a Dutch style auger in shaded and unshaded plots at the end of the shading period to compare the soil moisture content of the upper profile (gravimetric method was used (Rowell, 2014)). The level of reduction in photosynthetically active radiation (PAR) incident on the crop as a result of shading was measured by taking simultaneous measurements of PAR above the crop canopy under the shades and above the crop canopy in adjacent unshaded areas using a Sunscan Canopy Analysis System (Delta T Devices, Cambridge, UK). Crop height was measured throughout the shading period in the undisturbed pre-harvest sampling areas of treated and untreated areas by measuring the height of five randomly selected shoots from ground level to the uppermost leaf ligule or ear collar (if present). The percentage green area of whole treatment areas was estimated at approx. weekly intervals during the latter stages of canopy senescence in shaded 'late' and unshaded treatments. Leaning and lodging was assessed in each treatment area just prior to harvest with a whole treatment area % score given to each of the five categories: shoots upright; shoots leaning (0-5° from the vertical); shoots lodged (5°-45° from the vertical); lodged and flat (45°-90° from the vertical); brackled (stem failure of >1/4 or more up its length).

3.2.2.3 Destructive sampling

There was at least 0.5 m distance between adjacent sample areas which were at least 0.5 m from the ends and edges of plots/treatments. Tram lines and drill overlaps were also avoided with the aim of selecting sample areas that were representative of the plot.

Grain weight was assessed at individual grain locations, or zones, on ears at the beginning and end of treatment periods and again at harvest in both seasons. Additional intermediate weekly assessments were carried out during the treatment period in 2011. These detailed grain weight assessments were carried out on the main stem ear (MS) only in 2011, but also on two subsequent tiller ears (T1 and T2) in

2012 for unshaded, 'late' shading and 'early' shading treatments. In 2011 ten main stem ears per treatment within the designated sampling area were sampled at random on each sampling occasion (main stems were tagged with a small wire ring prior to the onset of tillering). In 2012 ten plants were sampled from the designated sampling area and the MS, T1 and T2 were identified based on their growing position at the plant base with decreasing stem diameter, height and ear length also used as indicators of tiller order if growing position was not clear. An ear was not sampled until it was at least 50% emerged. Ear samples were stored in sealed plastic bags in a cold room at 4-6 °C prior to sampling of individual grains.

The central grain on each ear was identified by counting the number of spikelets (fertile and infertile) upwards from the ear collar (alternating from one side of the ear to the other), halving the total number, and then rounding up to the next whole number. Grains were then sampled individually by location, or 'zone', with central grains holding zone 0, grains above +1, +2 etc. and grains below -1, -2 etc. Cultivar Quench is a two-row barley variety where only the median spikelets are fertile. A spikelet was defined as possessing a 'grain' once it had swollen to twice the width of the two lateral infertile spikelets or if it had developed an awn (see Figure 3.2) and was not sampled unless it satisfied these criteria. The grains were sampled by removing bulk florets (including lemma, palea and awn) from each zone and bulking zones across all 10 ears. The number of grains per zone was also counted so data would provide an accurate estimation of grain no. ear⁻¹. Material was then dried at 70 °C for 48 hours (or to a constant mass) before the dry weight was recorded to 0.1 mg. Harvest data was adjusted for the presence of non-grains by removing from data sets all grain dry weight values ≤ 2.9 mg (the mean weight of a non-grain identified in section 3.3.1.3.)

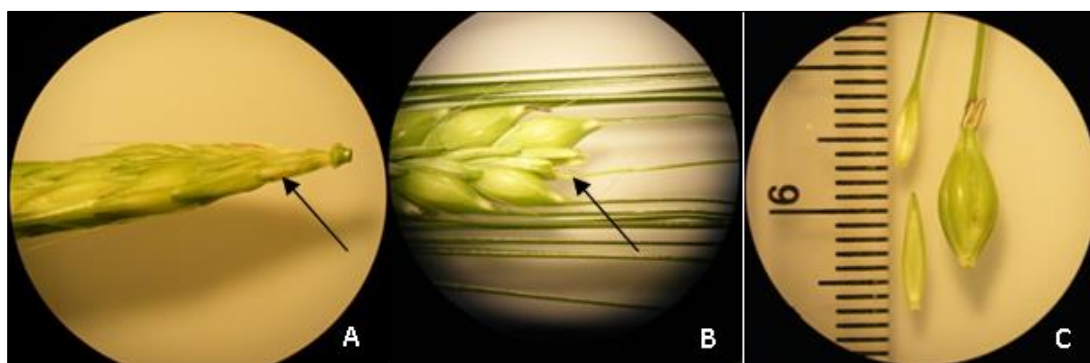


Figure 3.2. Examples of small grains at the base of the ear (A) and the tip of the ear (B) typically sampled as part of detailed grain weight assessments. These grains are shown alongside an additional larger central grain in (C). Graduations in mm. Photographs were taken at the late-milk to early-dough developmental stage.

At harvest ripeness, a quadrat sample size of 6 x 1 m undisturbed adjacent row lengths of above ground crop material (equating to 0.72 m²) was removed from each treatment area and air-dried prior to processing. A 40% sub-sample (by shoot number) was obtained for above ground dry matter determination and a further 20% was separated into ears and straw. Each portion was subject to dry matter determination and ear counts were also carried out. Ears were then hand-threshed between two pieces of foam board and sieved over a mechanically operated 1 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate into chaff and grain portions. Where chaff material remained in the grain portion on top of the sieve it was removed with tweezers and added to the chaff portion. Material was re-dried before the dry weight of each portion was recorded. MGW was also calculated for each plot using an automated grain counter (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate 25 g grain sample. After counting, grain weight was determined to the nearest 0.1 mg. Hand threshed grain yield (t ha⁻¹) was then expressed at 85 % dry matter, grain number m⁻² calculated using MGW and plot yield data, and grain number per ear calculated using grain number data and ear number data. These yield data were retrospectively adjusted for the presence of non-grains as per section 3.2.1.4.

Grain weight distribution histograms were produced for shaded 'late' and unshaded treatments in 2011 only as described in section 3.2.1.2.

Shading experiments were situated adjacent to the Carlow (CW) field experiments described in Chapter 2. As such, biomass data obtained from this adjacent experiment at the time when 'late' shading was applied were used to calculate the difference in accumulated biomass between GS 55 + 14 days and harvest in 'late' shaded and unshaded treatments.

3.2.2.4 Statistical analysis

Harvest yield, yield component, biomass and accumulated biomass data obtained from the pre-harvest quadrat samples were analysed statistically for effects of shading using ANOVA in GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK), with the relevant treatment structures described. Grain number per ear data from the detailed grain weight assessments was similarly analysed. Following ANOVA, relevant means for treatments of interest were compared using the standard error of the difference (S.E.D.) between means, on the residual degrees of freedom (d.f.) from the ANOVA, thus invoking the least significant difference (L.S.D.) at the $P = 0.05$ level of significance.

Repeated measures analysis was carried out on data from detailed grain weight assessments at harvest in all three seasons using GenStat. Correlations between individual zones were accounted for in the model used. Main stem data for 2011 and 2012 were first analysed to determine shading, zone, and shading x zone interaction effects on grain weight for main stems only. Due to missing values at ear extremities 2011 data were restricted to zones +14 to -11 and 2012 to zones +13 to -11. A further repeated measures analysis was carried out on 2012 data where tiller hierarchy was included as a factor. It was necessary to restrict data to zones +11 to -9 in this instance due to missing values at ear extremities particularly on later formed tillers where there were fewer grains per ear.

Data from weekly detailed grain weight assessments in 2011 were statistically analysed using a three way factorial repeated measures analysis (2 treatments x 7

sampling occasions x 28 zones) with SAS (Version 9.3, SAS Institute Inc., Cary, USA). A spatial structure was employed to handle two directions of correlation – grain weights from different sampling dates were correlated as were grain weights from different ear zones. The model used assumed a positive correlation. Taking into account possible variation within replicates the significance of the differences between ‘late’ shaded and unshaded was estimated to 95% confidence intervals. Only data from zones +15 to -12 were included in the analysis due to missing values above and below these zones, because not all 10 ears sampled had grains in zones above and below this range.

3.3 Results

3.3.1 Harvesting method and MGW

3.3.1.1 Comparison of harvest and threshing method across sites, seasons and seed rate treatments

There was no significant harvesting method effect on MGW ($P = 0.960$) for the nine site/seasons of data from Chapter 2 (Table 3.1); the MGW for each was 46 mg. There was also no significant site x season x harvesting method interaction ($P = 0.790$) and no significant site x harvesting method interaction ($P = 0.350$) for MGW. There was however a significant season x harvesting method interaction ($P = 0.005$) where combine-threshed MGW was 2.7 mg lower than hand-threshed MGW in 2013 but there was no difference in 2011 and 2012.

There was no significant harvesting method effect on MGW ($P = 0.317$) for the CW seed rate experiment in 2013 (Table 3.2). However at the KK site, there was a harvesting method effect on MGW ($P = 0.031$, Table 3.2) – combine threshed MGW was 1.5 mg lower than hand threshed MGW. There was a significant seed rate effect at both CW ($P = 0.002$) and KK ($P = 0.032$) but no significant seed rate x harvesting method interaction effect ($P = 0.692$ and 0.437 respectively).

Table 3.1. Mean values of mean grain weight (MGW, mg) expressed at 85 % dry matter (DM) along with P values and L.S.D. 5% (least significant difference at P = 0.05) values for main effects of harvesting method and various interaction effects following ANOVA on nine site/seasons of data. ns = non-significant.

Means		MGW, mg					
Harvesting method		combine	hand				
		46.44	46.41				
Season x harvesting method		combine	hand				
	2011	48.96	47.75				
	2012	44.03	42.44				
	2013	46.32	49.03				
Site x harvesting method		combine	hand				
	CK	43.81	44.52				
	CW	48.29	47.13				
	WX	47.21	47.57				
Site x season x harvesting method		CK	CK	CW	CW	WX	WX
		combine	hand	combine	hand	combine	hand
	2011	46.41	45.69	50.33	49.26	50.14	48.3
	2012	43.75	43.40	45.29	41.96	43.04	41.97
	2013	41.26	44.47	49.24	50.17	48.45	52.45
Effects							
	<i>P</i>	<i>L.S.D.</i> 5%	<i>d.f.</i>				
<i>Harvesting method</i>	0.960	ns	30				
<i>Season x harvesting method</i>	0.005	1.947	30				
<i>Site x harvesting method</i>	0.350	ns	30				
<i>Site x season x harvesting method</i>	0.790	ns	30				

Table 3.2. Mean values of mean grain weight (MGW, mg) expressed at 85 % dry matter along with *P* values and L.S.D. 5% (least significant difference at *P* = 0.05) values for main effects of harvesting method, main effects of seed rate, and harvesting method x seed rate interaction effects following ANOVA on data from the CW and KK seed rate experiments in 2013. ns = non-significant.

Means	MGW, mg CARLOW			MGW, mg KILKENNY		
Harvesting method	combine 48.51	hand 47.34		combine 48.31	hand 49.80	
Seed rate	40 seeds m ⁻²	53.00		40 seeds m ⁻²	51.32	
	80 seeds m ⁻²	50.68		80 seeds m ⁻²	49.54	
	160 seeds m ⁻²	45.94		160 seeds m ⁻²	49.11	
	320 seeds m ⁻²	46.32		320 seeds m ⁻²	48.87	
	640 seeds m ⁻²	45.86		640 seeds m ⁻²	47.35	
	1280 seeds m ⁻²	45.75		1280 seeds m ⁻²	48.13	
Seed rate x harvesting method		combine	hand		combine	hand
	40 seeds m ⁻²	53.92	52.07	40 seeds m ⁻²	50.31	52.33
	80 seeds m ⁻²	51.15	50.22	80 seeds m ⁻²	48.78	50.31
	160 seeds m ⁻²	48.58	43.31	160 seeds m ⁻²	49.59	48.63
	320 seeds m ⁻²	45.95	46.69	320 seeds m ⁻²	47.13	50.61
	640 seeds m ⁻²	45.87	45.84	640 seeds m ⁻²	47.13	47.58
	1280 seeds m ⁻²	45.61	45.90	1280 seeds m ⁻²	46.93	49.33
Effects	CARLOW			KILKENNY		
	<i>P</i>	<i>L.S.D.</i> 5%	<i>d.f.</i>	<i>P</i>	<i>L.S.D.</i> 5%	<i>d.f.</i>
Harvesting method	0.317	ns	31	0.031	1.342	33
Seed rate	0.002	4.074	31	0.032	2.325	33
Seed rate x harvesting method	0.692	ns	31	0.473	ns	33

3.3.1.2 Grain weight distribution

Grain weight distribution histograms for combine-threshed (Figure 3.3 (a) and (c)) and hand-threshed (Figure 3.3 (b) and (d)) grain samples from CW and WX experimental sites in 2011 are largely similar in appearance. The only apparent difference is that hand-threshed samples have more grains in the 0-2.5 mg class at CW and the < 5.0 mg classes at WX.

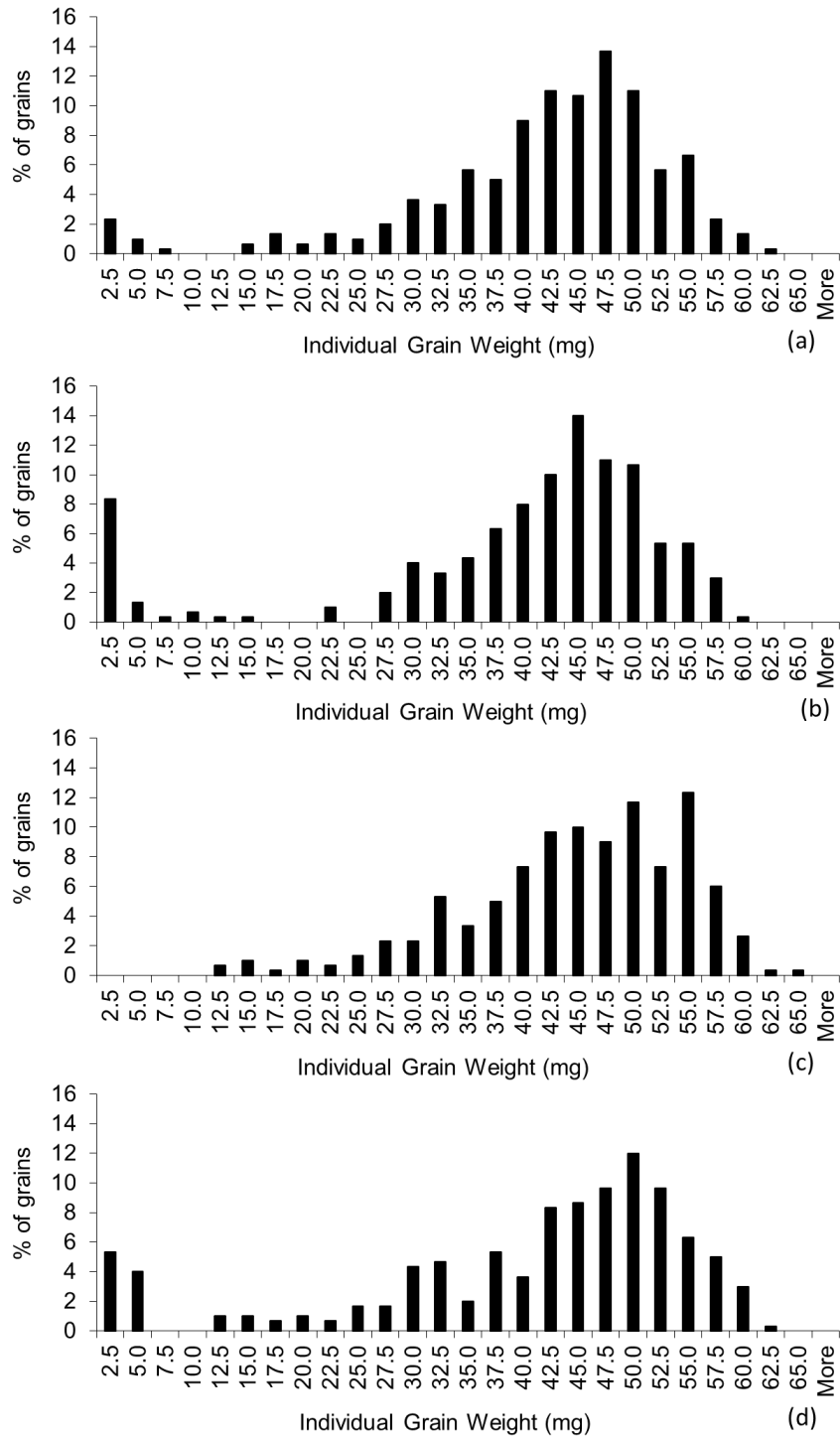


Figure 3.3. Grain weight distribution histograms at harvest for (a) CW 2011 combine sample, (b) CW 2011 hand-threshed grain sample, (c) WX 2011 combine grain sample and (d) WX 2011 hand-threshed grain sample; $n = 300$. Grain weight is expressed at 100% dry matter.

3.3.1.3 Vital staining

Test staining showed that viable seeds had a uniform red staining on the embryo, whilst endosperms were unstained and thus easily distinguishable also. Non-viable grains had neither an embryo nor an endosperm (Figure 3.4).

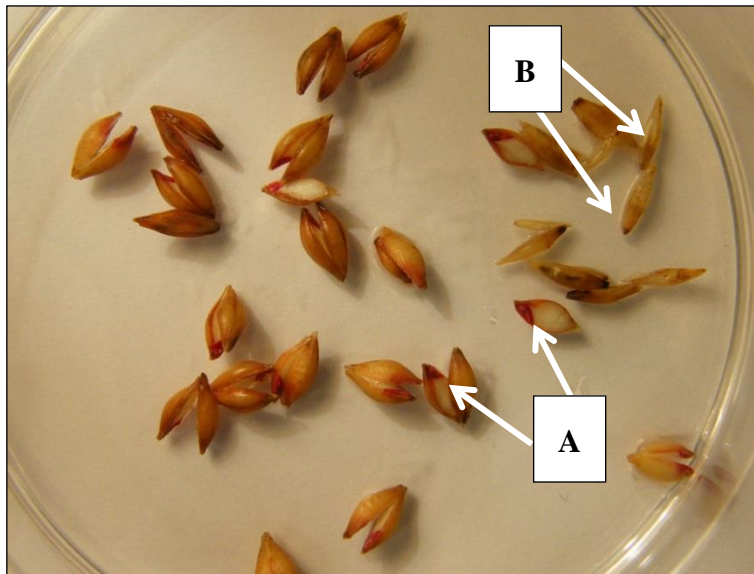


Figure 3.4. Test staining of grains with 2,3,5 triphenyltetrazolium chloride (TTC) solution. Viable grains showing a stained embryo and unstained endosperm (A) non-viable grains had neither (B).

Most viable grains, by this definition, were visually distinguishable without TTC staining in a hand-threshed grain sample as full, rounded and hard. Doubt remained over very small, slim ‘grains’ as to whether they had a very small indistinguishable embryo and endosperm or were simply empty lemma and palea. Sieving of hand-threshed samples showed that these suspect grains lay in the 1 mm – 1.75 mm size class. All grains in the >1.75 mm size class were visually distinguishable as grains and all material in the < 1 mm size class was visually identifiable as chaff.

Staining of grains in the 1 mm – 1.75 mm size class of hand-threshed grain samples

from the KK seed rate experiment in 2013 enabled the number of viable grains (those with a clearly identifiable embryo and endosperm) and non-viable grains (everything else) to be quantified (Table 3.3). The number of non-grains m^{-2} increased with seed rate ($P = 0.002$). However seed rate also had a significant effect on ear number m^{-2} ($P < 0.001$) resulting in no significant effect of seed rate on the number of non-grains per ear ($P = 0.884$) the mean value of which was 0.45 non-grains per ear. The relationship between ear number m^{-2} and the number of non-grains m^{-2} was plotted and an exponential curve proved to be a marginally better fit ($R^2 = 0.69$) than a straight line regression ($R^2 = 0.65$). The mean dry weight of a non-grain was estimated at 2.9 mg from dried non-grain material identified from the staining procedure.

Table 3.3. Mean values of the number of non-grains m^{-2} , ear number m^{-2} and the number of non-grains per ear at each seed rate following vital staining on grain samples from the KK 2013 seed rate experiment. P values and L.S.D. 5% (least significant difference at $P = 0.05$) values are for main effects of seed rate following ANOVA. ns = non-significant.

Mean values	No. non-grains m^{-2}	Ear no. m^{-2}	No. non-grains ear ⁻¹
40 seeds m^{-2}	273	538	0.473
80 seeds m^{-2}	285	629	0.473
160 seeds m^{-2}	302	856	0.393
320 seeds m^{-2}	368	940	0.401
640 seeds m^{-2}	515	1260	0.463
1280 seeds m^{-2}	703	1603	0.477
<i>Seed rate effects</i>	<i>No. non-grains m^{-2}</i>	<i>Ear no. m^{-2}</i>	<i>No. non-grains ear⁻¹</i>
<i>P</i>	<i>0.002</i>	<i><.001</i>	<i>0.884</i>
<i>L.S.D. 5%</i>	<i>197</i>	<i>183</i>	<i>ns</i>
<i>d.f.</i>	<i>15</i>	<i>15</i>	<i>15</i>

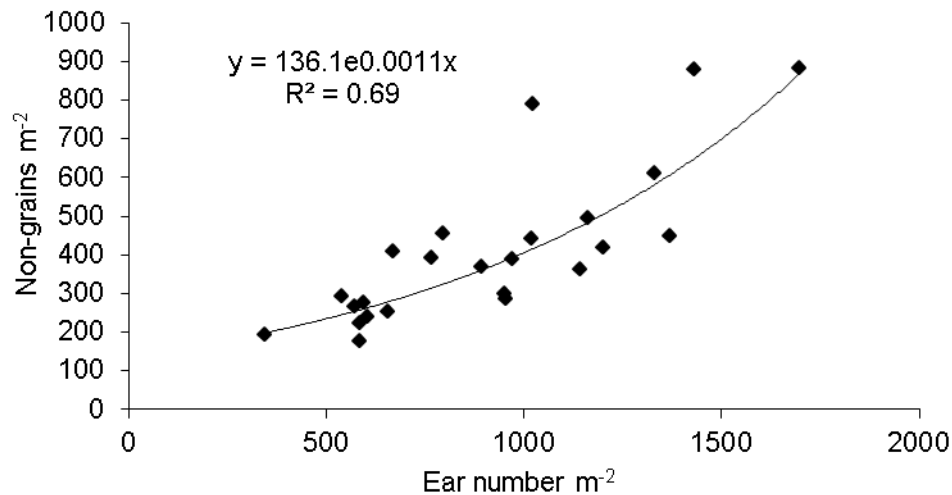


Figure 3.5. An exponential curve fitted to plot of ear number m^{-2} versus non-grains m^{-2} estimated from grain staining of KK 2013 harvest samples. Line fitted using Microsoft Excel (Microsoft Office 2010, Microsoft Corporation, Washington, USA).

3.3.2 Responses to post-anthesis shading

Crops established well in both seasons - percentage plant establishment from the seed rate of 330 seeds m^{-2} was 89 % and 96 % in 2011 and 2012 respectively. Further climate and site information for the CW site in 2011 and 2012 is given in Chapter 2, section 2.3.1.

3.3.2.1 Micro-climate, crop development, leaning and lodging

The mini-meteorological stations installed in shaded and unshaded plots in 2011 showed that the temperature just above the canopy was on average 0.4 °C cooler in the shaded plots than the unshaded plots and the relative humidity was on average 0.3 % higher in the shaded plots (hourly data averaged across the whole 'late' shading treatment period of 52 days). Percentage soil moisture in the 0 – 30 cm profile at the end of the shading period in 2011 was 21% for the shading treatment and was significantly higher than the unshaded control value of 15 % ($P = <0.001$); there was no difference in 2012. Shading had no significant effect on crop height in

either season. PAR reduction due to shading was consistent on each measurement occasion with average reductions of 59 % in 2011 and 2012.

There were significant effects of ‘late’ shading on stem brackling ($P = 0.002$) and leaning ($0-5^\circ$ from the horizontal, $P = 0.038$) at harvest in 2011 (Table 3.4). Brackling occurred on 12% of stems for the ‘late’ shading treatment compared to 2% for unshaded controls and leaning occurred on 4% of stems compared to 1% for controls. No shoots were lodged ($5-45^\circ$ from the horizontal) in 2011. There was a significant effect of shading on leaning only at harvest in 2012 ($P = 0.046$) where ‘early’ shading reduced the amount of leaning that occurred - 60% compared to 85% for the unshaded control (Table 3.4). While lodging and brackling occurred in 2012 there was no significant difference between treatments. The leaning, lodging and brackling mentioned occurred in both seasons after Zadoks GS 87 (Tottman, 1987) when grain filling had been completed. No shoots were lodged flat ($45^\circ-90^\circ$) in any treatment or season.

Table 3.4. Mean values of % of shoots leaning, lodged, and brackled for shaded and unshaded treatments at harvest in 2011 and 2012. Values are means of % scores for the whole treatment area. P values are for effects of shading following ANOVA. ns = non-significant. No shoots were lodged flat ($45^\circ-90^\circ$) in either season.

Mean values	2011		2012		
	Shaded late	Unshaded	Shaded early	Shaded late	Unshaded
Shoots leaning ($0-5^\circ$)	4	1	60	85	85
Shoots lodged ($5-45^\circ$)	0	0	14	4	4
Shoots brackled	12	2	9	8	1
Shading effects	2011		2012		
	P	d.f.	P	L.S.D. 5%	d.f.
Shoots leaning ($0-5^\circ$)	0.038	5	0.046	22	6
Shoots lodged ($5-45^\circ$)	-	5	0.348	ns	6
Shoots brackled	0.002	5	0.066	ns	6

A delay in canopy senescence was observed in ‘late’ shaded plots compared to unshaded controls in 2011 and 2012. This was quantified during the latter stages canopy senescence and data are illustrated in Figure 3.6. The pattern of senescence for ‘early’ shaded plots was similar to unshaded controls.

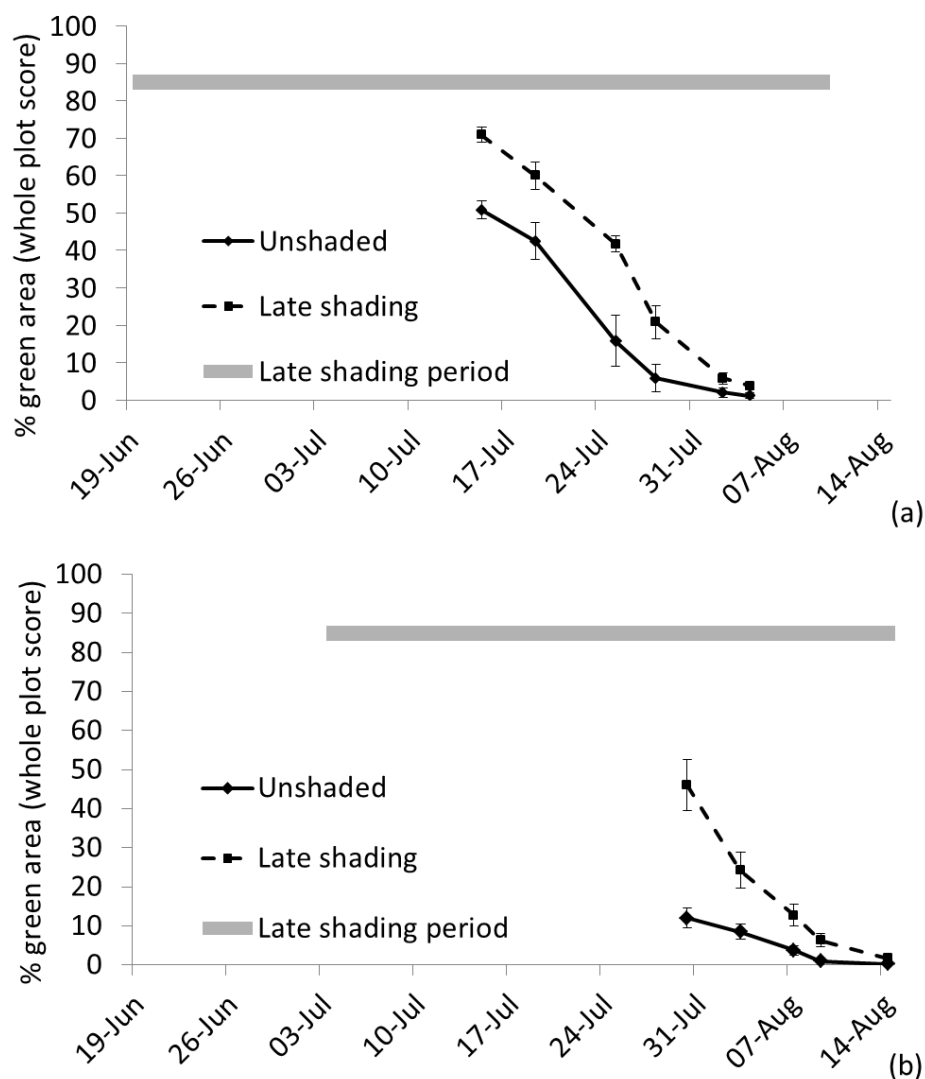


Figure 3.6. Canopy senescence for unshaded and ‘late’ shading treatments in 2011 (a) and 2012 (b). Values are means \pm SEM of replicate values of whole treatment area % green area scores at a range of dates throughout the latter stages of senescence. The periods of late shading in each season are also shown as a shaded bar.

3.3.2.2 Yield and yield components

While there were consistent reductions in mean grain number m^{-2} for all shading treatments, none were statistically significant ($P > 0.05$; Table 3.5). Also, neither of the two grain number sub-components (ear number m^{-2} and grain number per ear) were significantly reduced by shading. ‘Late’ shading significantly reduced yield, MGW and harvest index in 2011 and 2012, whilst ‘early’ shading in 2012 had no significant effect on yield or MGW but did significantly increase harvest index (Table 3.5). Across all treatments and seasons, yield reductions due to shading ranged from 8% – 20% and significant MGW reductions from 3% - 12%.

Total biomass and ear biomass at harvest were reduced by ‘late’ shading in 2011 and 2012 ($P < 0.05$, Table 3.5). ‘Late’ shading reduced the amount of total biomass accumulated during the treatment period in both 2011 ($P = 0.039$) and 2012 ($P = 0.049$) by 28% and 52% respectively in response to a 59% PAR reduction in both seasons. There was no significant effect of ‘early’ shading on total and ear biomass at harvest in 2012 and there was no significant effect of any shading treatment on straw and leaf biomass values at harvest. It was not possible to estimate biomass accumulated during shading for the ‘early’ shading treatment in 2012 as quadrat data at the beginning of the treatment were not available.

Table 3.5. Mean values of yield, yield components and other harvest variables for shaded and unshaded treatments in 2011 and 2012. *P* values and *L.S.D.* 5% (least significant difference at *P* = 0.05) values are for effects of shading following ANOVA; *ns* = non-significant; *DM* = 100% dry matter; yield and *MGW* are expressed at 85% *DM*

	Mean values 2011		Shading effects 2011		Mean values 2012			Shading effects 2012		
	Shaded late	Unshaded	<i>P</i>	<i>d.f.</i>	Shaded early	Shaded late	Unshaded	<i>P</i>	<i>L.S.D.</i> 5%	<i>d.f.</i>
Yield (t ha ⁻¹)	8.84	10.98	0.025	5	7.34	6.45	7.98	0.026	0.99	6
Grain number m ⁻²	21266	22347	0.550	5	18361	18685	20335	0.253	<i>ns</i>	6
<i>MGW</i> (mg)	47.72	49.33	0.001	5	40.05	34.47	39.33	0.033	4.17	6
Ear number m ⁻²	1156	1208	0.531	5	935	892	997	0.168	<i>ns</i>	6
Grain number ear ⁻¹	18.4	18.43	0.947	5	19.69	21.02	20.39	0.623	<i>ns</i>	6
Harvest Index	56.14	59.90	0.005	5	53.15	48.42	50.87	0.004	2.07	6
Ear number plant ⁻¹	3.9	4.1	0.531	5	2.9	2.8	3.1	0.168	<i>ns</i>	6
Total biomass (t ha ⁻¹ <i>DM</i>)	13.71	15.68	0.039	5	11.98	11.19	13.35	0.049	1.66	6
Ear biomass (t ha ⁻¹ <i>DM</i>)	9.12	11.09	0.038	5	7.39	6.68	8.18	0.026	0.97	6
Straw and leaf biomass (t ha ⁻¹ <i>DM</i>)	4.77	5.01	0.552	5	4.85	4.84	5.59	0.067	<i>ns</i>	6
Biomass acc. during shading (t ha ⁻¹ <i>DM</i>)	5.13	7.10	0.039	5	-	1.97	4.13	0.004	0.82	6

Grain number per ear data obtained from the detailed grain weight assessments at harvest (Table 3.6) show that neither ‘early’ nor ‘late’ shading significantly reduced grain no. ear⁻¹ in either season. There was a significant tiller effect on grain no. ear⁻¹ in 2012 ($P = < 0.001$) where tillers produced later had fewer grains per ear but this was not accompanied by a shading x tiller interaction effect. This implies that shoots of contrasting hierarchy did not differ in sensitivity to shading.

Table 3.6. Effects of shading treatments on grain number per ear for main shoots (MS) in 2011 and main shoots, tiller 1 (T1) and tiller (2) in 2012. P values and L.S.D. 5% (least significant difference at $P = 0.05$) values are for effects of shading, tiller hierarchy and shading x tiller hierarchy interaction effects following ANOVA; ns = non-significant.

Mean values	2011		2012		
	Shaded late	Unshaded	Shaded early	Shaded late	Unshaded
Grain number ear ⁻¹	24.15	24.37	21.39	21.73	22.01
			MS	T1	T2
Grain number ear ⁻¹			24.11	21.21	19.82
Effects	2011		2012		
	<i>P</i>	<i>d.f.</i>	<i>P</i>	<i>L.S.D.5%</i>	<i>d.f.</i>
Shading	0.698	5	0.244	ns	ns
Tiller hierarchy			<.001	0.801	6
Shading x tiller hierarchy			0.912	ns	ns

3.3.2.3 Grain growth and location on the ear

Grains in central zones on the ear were heavier than those in upper distal and lower basal zones in the unshaded control throughout the ‘late’ shading treatment period in 2011 (Figure 3.7). This was most pronounced at harvest where grain weight was heaviest in the approximate zones -1 to -6. The decline in grain weight beyond these zones was steeper towards the base of the ear than it was towards the top. In 2011, measurable differences in grain growth were first observed in central ear zones two weeks into the shading treatment (GS 55 + 28 days). There is some evidence in later assessments and at the harvest assessment that grain growth in distal zones was also reduced by shading to a level comparable to that of grain in central zones, however due to increasing standard error and variance towards the upper and lower extremities of the ear the data become less reliable in these zones. The weight of grains at all zones increased steadily throughout the grain filling period under shaded and unshaded conditions. There was no evidence that grain growth at any zone ceased after shading, or that grain weight decreased as might occur if grains aborted and material was remobilised and recycled. The pattern of grain weight at individual zones at the onset of shading in 2012 (GS 55 + 14 days) was similar to that of 2011. At harvest in 2012, the pattern of grain growth and grain weight reduction due to shading was similar to harvest 2011, however grain weight was slightly depressed across all zones in shaded and unshaded treatments compared to 2011.

The statistical significance of the differences in weight at individual ear zones between ‘late’ shaded and unshaded treatments in 2011 are shown in Table 3.7 in support of visual evidence presented in Figure 3.7. The first significant reductions of shading on grain weight were noticed in central zones of the ear 14 days into the shading period at GS 55+28 days. These effects became statistically stronger with time and appear to spread outwards to more distal grains. At harvest, grain weight at all zones included in the analysis bar the most extreme upper distal zones was significantly affected.

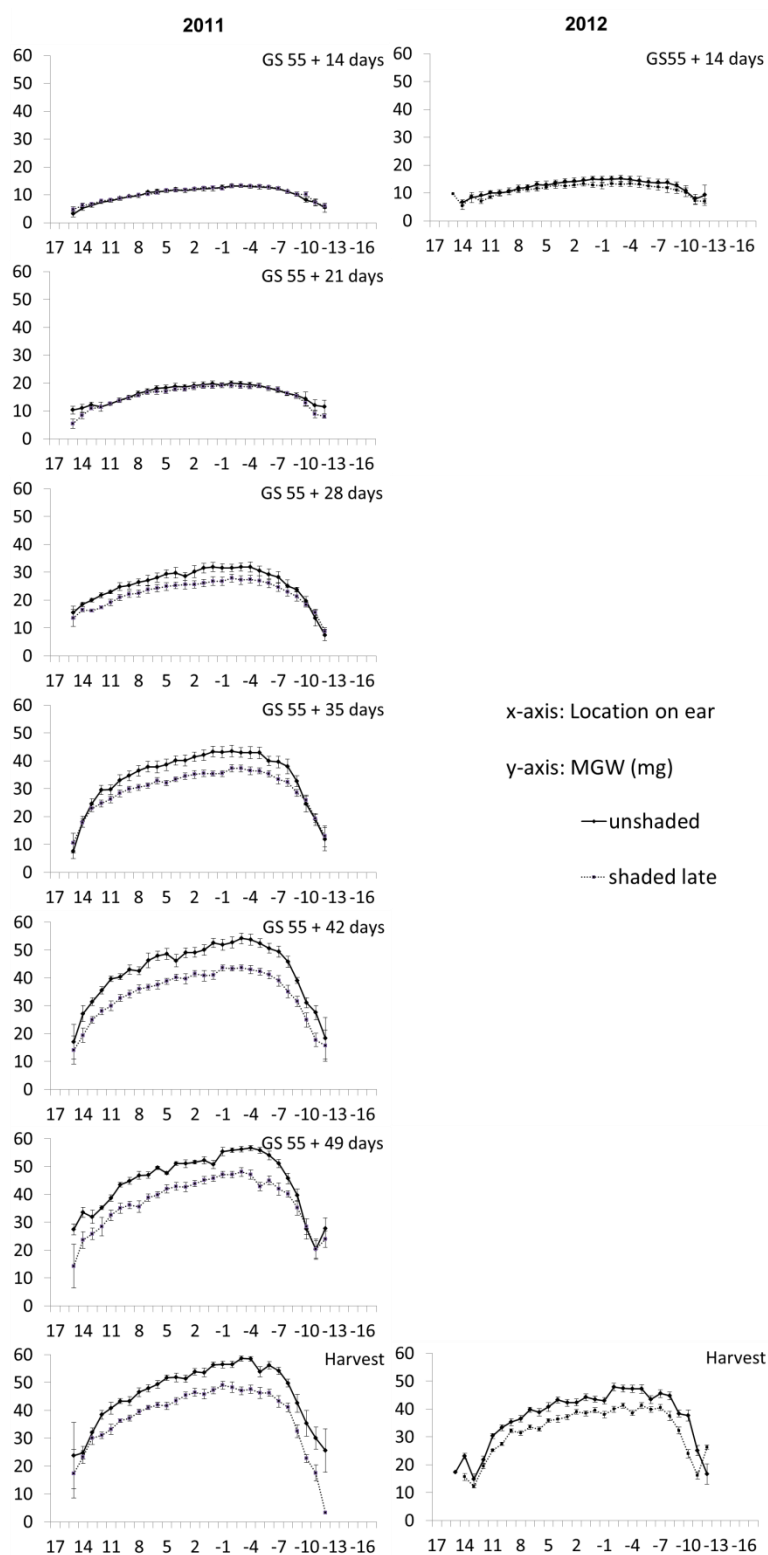


Figure 3.7. Plots of grain weight at individual zones on main stem ears for unshaded and 'late' shading treatments in 2011 and 2012. Values are means \pm SEM of ten ears. MGW is expressed at 100% dry matter (DM) and includes the weight of the lemma and palea.

*Table 3.7. Effects of 'late' shading on MGW at individual grain locations on the ear and at weekly intervals post treatment until harvest in 2011. Data were analysed statistically using a three way factorial repeated measures analysis of variance (2 treatments x 7 sampling occasions x 28 zones). ns = non-significant; The significance of the differences between 'late' shaded and unshaded was estimated to 95% confidence intervals * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.*

2011	GS 55+14 days	GS 55+21 days	GS 55+28 days	GS 55+35 days	GS 55+42 days	GS 55+49 days	Harvest
15	ns	ns	ns	ns	ns	ns	ns
14	ns	ns	ns	ns	**	*	ns
13	ns	ns	ns	ns	**	ns	ns
12	ns	ns	*	*	***	**	**
11	ns	ns	ns	ns	***	**	***
10	ns	ns	*	*	***	***	***
9	ns	ns	ns	*	***	***	**
8	ns	ns	*	**	***	***	***
7	ns	ns	ns	***	***	***	***
6	ns	ns	ns	*	***	***	***
5	ns	ns	*	***	***	**	***
4	ns	ns	*	***	***	***	***
3	ns	ns	ns	**	***	***	**
2	ns	ns	*	**	***	***	***
1	ns	ns	**	***	***	***	***
0	ns	ns	*	***	***	*	***
-1	ns	ns	*	***	***	***	***
-2	ns	ns	ns	**	***	***	***
-3	ns	ns	*	**	***	***	***
-4	ns	ns	*	**	***	***	***
-5	ns	ns	ns	**	***	***	***
-6	ns	ns	ns	*	***	***	***
-7	ns	ns	ns	**	***	***	***
-8	ns	ns	ns	**	***	*	***
-9	ns	ns	ns	*	***	ns	***
-10	ns	ns	ns	ns	**	ns	***
-11	ns	ns	ns	ns	***	ns	***
-12	ns	ns	ns	ns	ns	ns	***

As stated, only 'late' shading had a significant effect on MGW at harvest (Table 3.5). Repeated measures analysis of grain weight data at individual grain zones on main stem ears at harvest ripeness in the two seasons where 'late' shading was applied

(Table 3.8) confirms this shading effect in both seasons ($P < 0.001$ in 2011 and $P = 0.008$ in 2012). There was also a significant zone effect on grain weight in 2011 ($P = 0.001$) and 2012 ($P = 0.001$) where central grains of the ear were heavier than those in more distal zones (Figure 3.7). There was no significant shading x zone interaction effect in 2011 ($P = 0.230$) or 2012 ($P = 0.414$) indicating that effects of shading on grain weight were the same irrespective of the grain's location on the ear.

Table 3.8. Effects of shading, grain zone and shading x grain zone interaction effects following a repeated measures ANOVA on main stem ear grain weight data at individual grain zones at harvest ripeness in 2011 and 2012. LSD 5% = least significant difference at $P = 0.05$

Year	Effects	P	L.S.D. 5% (mg)	d.f.
2011 (Zones 14 to -11)	Shading	< 0.001	1.55	5
	Grain zone	< 0.001	3.73	31
	Shading x grain zone	0.230	5.37	34
2012 (Zones 13 to -11)	Shading	0.008	2.92	6
	Grain Zone	< 0.001	3.81	30
	Shading x grain zone	0.414	7.06	37

When tiller hierarchy was included as a factor in the repeated measures analysis for 2012 (Table 3.9), there was a significant tiller effect on grain weight ($P = < 0.001$) where the main stem (MS) MGW of 39.05 mg was significantly higher than tiller 1 (T1) and tiller 2 (T2) MGW's of 34.65 and 33.25 mg respectively. There was a significant zone x tiller interaction effect ($P < 0.001$) on grain weight (Figure 3.8) – grain weights on the MS were comparable to those of T1 and T2 in central locations on the ear but greater in upper (distal) and lower (basal) positions. There was no significant shading x tiller interaction effect ($P = 0.217$) and no significant shading x tiller x zone three-way interaction effect ($P = 0.824$) on MGW, confirming that shoots of contrasting hierarchy did not differ in sensitivity to shading on either a whole ear or zone basis.

Table 3.9. Effects of shading, zone, tiller hierarchy and associated interaction effects following a repeated measures ANOVA on grain weight data at individual grain zones from MS, T1 and T2 ears in 2012. L.S.D. 5% = least significant difference at $P = 0.05$

Year	Effects	P	L.S.D. 5% (mg)	d.f.
2012 (Zones 11 to -9)	Shading	< 0.001	1.63	24
	Grain zone	< 0.001	2.72	155
	Shading x grain zone	0.328	ns	179
	Tiller hierarchy	< 0.001	1.63	24
	Grain zone x tiller hierarchy	< 0.001	4.96	179
	Shading x tiller hierarchy	0.217	ns	24
	Shading x tiller hierarchy x grain zone	0.824	ns	179

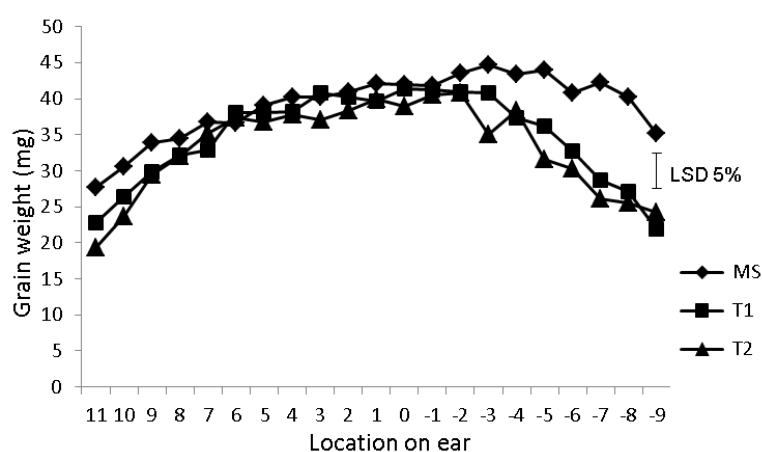


Figure 3.8. Grain weight on MS, T1 and T2 ears at individual grain locations (zones) at harvest in 2012. Data are means of the three shading treatments in 2012 (unshaded, 'early' shading, and 'late' shading). Error bar is L.S.D. 5% (least significant difference at $P = 0.05$) for grain zone x tiller interaction effect following repeated measures analysis.

Evidence of 'late' shading effects on the grain weight distribution of harvest samples in 2011 is given in (Figure 3.9). The distribution is reduced to lower weight classes with 'late' shading however there does not appear to be any major change in the overall shape of the distribution nor are there any additional grains in the non-viable class ≤ 2.9 mg.

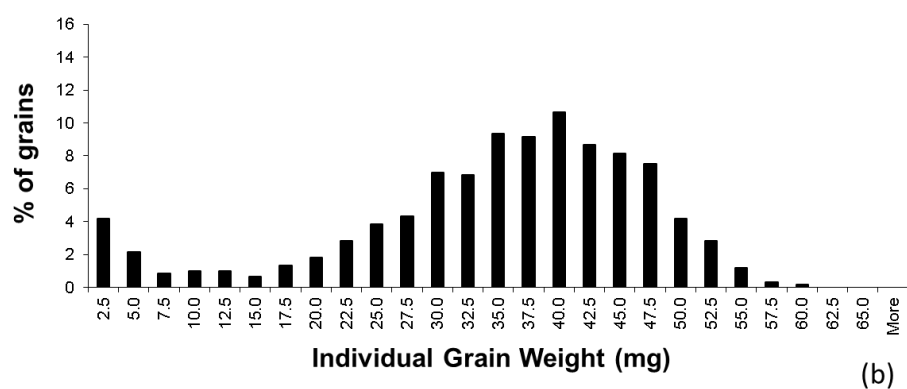
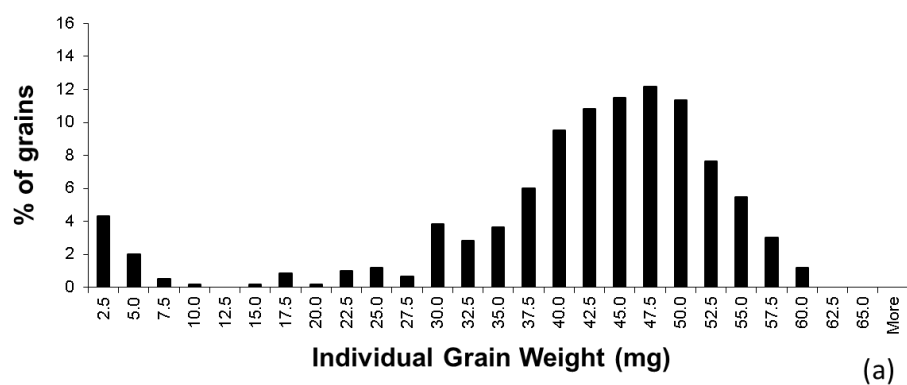


Figure 3.9. Grain weight distribution histograms at harvest for (a) unshaded and (b) 'late' shading treatments in 2011, $n = 600$. Grain weight is expressed at 100% dry matter.

3.4 Discussion

3.4.1 Harvesting method and MGW

A higher MGW in combine-harvested samples would be expected if lighter grain material was consistently lost from the grain sample however there was no evidence of a harvesting method effect on MGW across the 9 site/seasons of data. There was also no significant effect of harvesting method on MGW in the CW seed rate experiment in 2013 but there was at the KK experiment. However, this effect at KK in 2013 was in the opposite direction of what would be expected if combines were losing small grains. Combine harvested calculations of MGW were not consistently higher than hand harvested estimates of MGW therefore it is unlikely that the apparent conservation of MGW is a result of the loss of small grains from combine harvesters. Gallagher et al. (1975) also showed no consistent difference in MGW values calculated from hand-threshed and combine-threshed samples over three site/seasons in the UK. Mean grain number was 68 % higher for the nine site/seasons of data in the present study. Despite such a high yield potential where the proportion of small grains could potentially be greater due to increased competition for assimilate during grain filling, there was still no evidence of a harvesting method effect on MGW.

A comparison of grain weight distribution histograms showed that when compared to hand-threshed samples, combine-threshed samples retained most grains except those in the extreme lower weight classes (< 5.0 mg). Given the estimated mean non-grain dry weight of 2.9 mg obtained from vital staining of KK 2013 samples, it is unlikely that a large proportion of these additional ‘grains’ retained in hand-threshed samples were viable i.e. having acted as a sink post-anthesis.

Results of vital staining on KK 2013 seed rate experiment samples showed that the $1\text{ mm} > 1.75\text{ mm}$ grain size class contained both grain and non-grain material. While sieving hand-threshed grain samples over a 1 mm sieve was a reliable method of retaining all potential grains, the presence of non-grains in these samples remained an issue. A means of adjusting hand-threshed yield component data for the presence

of non-grains in samples was required. Scott et al. (1983) estimated that the weight of an empty husk at anthesis is 3 mg. The mean non-grain weight of 2.9 mg identified from vital staining was close to this value. From the vital staining, it appeared that there was a greater number of non-grains m^{-2} at higher seed rates but this was due to the fact that there was a greater ear number m^{-2} at higher seed rates – the number of non-grains per ear remained stable across all seed rate treatments at 0.45 per ear. Due to the strong influence that ear number m^{-2} had on the number of non-grains m^{-2} it was decided that a unique reduction factor would be calculated based on the ear number of the plot/treatment/crop in question and combined with the mean weight of a non-grain to retrospectively adjust hand-threshed yield component data throughout this thesis to account for the presence of non-grains. Grain number m^{-2} , mean grain weight (MGW), yield and grains per ear data calculated from hand-threshed grain samples sieved over a 1 mm sieve would all need to be either increased or reduced by this adjustment as per the method outlined in section 3.2.1.4.

3.4.2 Responses to post-anthesis shading

There was no evidence from any shading treatment of a significant reduction in grain number, ear number or grain number per ear in response to shading suggesting that following fertilisation and early development grains are unlikely to abort even if subject to large reductions in assimilate availability. Post-anthesis tiller death occurred at several site/seasons of experimentation discussed in Chapter 2. It is possible for some of the carbon and nitrogen assimilated by non-surviving shoots to move into the rest of the plant (Thorne, 1962; Thorne and Wood, 1987) and potentially buffer the effects of shading. However, as already stated, there was no evidence from any shading treatment of a significant reduction in ear number at harvest, therefore the pool of assimilate available for grain filling was unlikely to be altered to a lesser or greater extent in shaded crops. Also, no significant shading x tiller interaction effect on grain number per ear in 2012 indicates that a grain abortion mechanism was not more likely to occur on later formed tillers. The grain numbers achieved for the unshaded treatments in 2011 and 2012 of 22,347 and 20,335 grains

m^{-2} respectively were high in comparison to the mean value of 18,419 grains m^{-2} from the 9 site/season of data described in Chapter 2. As such, assimilate availability per grain prior to shading was already likely to be at the lower limit of what is practically achievable in the field, yet following a 59% reduction in solar radiation for an extensive post-anthesis period there was still no significant reduction in grain number per ear compared to unshaded controls.

Evidence presented contradicts some of the previous studies in barley and other crops where significant adjustments in grain number were observed following post-anthesis modifications in assimilation capacity (Boyer and McLaughlin, 2007; Boyer and Westgate, 2004; Estrada-Campuzano et al., 2008; Grashoff and d'Antuono, 1997; Habgood and Uddin, 1983; Nicolas et al., 1985; Westgate and Boyer, 1986; Zinselmeier et al., 1999). Post-fertilisation abortion of ovaries in maize following water deficits has been shown to be triggered by the depletion of ovary sugar pools (Boyer and McLaughlin, 2007; Boyer and Westgate, 2004; Zinselmeier et al., 1999). However these water deficit treatments were applied closer to pollination than the 'late' shading treatment in this study. Florets are particularly sensitive to environmental stress during meiosis (nuclear and cell division in preparation for anthesis) (Kirby and Appleyard, 1984) and environmental stress during early reproduction can result in abortion, sterility or decreased grain set (Fabian et al., 2011; Nicolas et al., 1985; Saini and Westgate, 2000). Given that anthesis does not occur simultaneously across all plants, ears, and spikelets of barley in a field context it was anticipated that the 'late' shading timing from 14 days after anthesis would allow all potential grains to be fertilised prior to shading. In such a scenario any down regulation of grain number m^{-2} in response to post-anthesis shading could be attributed to a post-anthesis abortion rather than non-fertilisation. When water deficits were applied to maize for a period similar to the 'late' shading period by (McPherson and Boyer, 1977) and (Jurgens et al., 1978) grain weight was reduced but there was little effect on grain number. This is in line with the present study and implies that grain abortion in response to a restricted assimilate availability may be more likely to occur closer to anthesis. However, the 'early' shading treatment in 2012 also resulted in no significant effect on grain number m^{-2} or grain number per ear.

Differences in temperature and relative humidity for shaded ‘late’ and unshaded environments were small. Shading did not reduce soil moisture in the surface soil layer in any treatment or season indicating that shading structures did not obstruct rainfall penetration to the crop. In fact, shaded treatments appeared to retain more moisture in the surface soil layer than in unshaded controls in 2011. This was likely due to reduced evapotranspiration in the shaded treatments. Given the small scale nature of the shading structures and that there was no evidence of drought in unshaded treatments or water logging in shaded treatments it was unlikely that these differences in soil moisture were important in terms of influencing yield and yield component values at harvest. Yield loss was expected to be negligible from any leaning or brackling effects of shading as they occurred post-grain-filling and were not severe enough to encourage sprouting of the grains or to prevent harvesting of any ears. As shown by Bingham et al. (2013) and Fisher (1975), the effects of shading on yield and its components were unlikely to be a consequence of changes in meteorological conditions other than the reduction in PAR.

There was however a delay in canopy senescence in shaded treatments compared to unshaded treatments perhaps as a consequence of the increased moisture retention and/or reduced light intensity in the shaded crops. While the prolonged green area may have increased the assimilatory capacity of the shaded crops somewhat towards the end of grain filling and as such buffered grain growth against the effects of shading, this difference appears to have been insufficient to offset the large reduction in PAR afforded. Biomass accumulated during shading was significantly less in shaded treatments, and both yield and MGW were significantly reduced by ‘late’ shading in 2011 and 2012 confirming a reduction in assimilatory capacity of the crops.

However, relative reductions in yield and MGW in response to shading were considerably lower than the PAR reductions afforded by shading. These yield and MGW reductions were also less, in relative terms, than the reduction in accumulated biomass during the shading period. Similar observations have been made by various other authors from similar shading experiments (Arisnabarreta and Miralles, 2008a; Grashoff and dAntuono, 1997; Serrago et al., 2013; Willey and Holliday, 1971).

These authors also found no significant reduction in grain number in response to shading with the exception of Grashoff and d'Antuono (1997) where a small reduction in final ear number has been reported. Photosynthate partitioning in shaded crops may have been modified to favor yield organs in the face of carbohydrate shortage (Fisher, 1975). Also, compensation mechanisms such as increased translocation of stored carbohydrates may have counteracted some of the effects of post-anthesis shading (Grashoff and d'Antuono, 1997; Scott et al., 1983). The importance of stem storage reserves in buffering grain growth and development have been demonstrated in barley (Grashoff and d'Antuono, 1997; Nosberger and Thorne, 1965; Serrago et al., 2013), wheat (Beed et al., 2007; Bell and Incoll, 1990; Blum et al., 1994; Davidson and Chevalier, 1992; Ehdaie et al., 2006; Fabian et al., 2011; Foulkes et al., 2007) and maize (McPherson and Boyer, 1977) and rice (Yang et al., 2001). The increased leaning and brackling observed in some shading treatments may be a consequence of such an increased utilisation of stem storage reserves whereby fewer carbohydrates were available for the formation of stem structural material.

The relative reduction in total accumulated biomass in shaded crops was less than the relative reduction in PAR, particularly in 2011. This suggests that RUE may have increased in shaded crops compared to unshaded ones offsetting some of the reduction in incident PAR and hence PAR interception (Bingham et al., 2007a; Calderini et al., 1997; Miralles and Slafer, 2006; Reynolds et al., 2005). Several factors could have contributed to this apparent increase in RUE. Upper leaves of unshaded canopies may have been light saturated, particularly in 2011 when higher than average levels of solar radiation post-anthesis were experienced. Shading may have reduced the degree of saturation. In addition, the delayed leaf senescence and prolonged canopy PAR interception in shaded treatments could have helped maintain photosynthesis for longer. Also, a reduction in partitioning of assimilate to the root system may have resulted in relatively more of the biomass being accumulated in above-ground tissues.

Even though the reduction in biomass accumulation was less than the reduction in PAR, 'late' shading significantly reduced MGW at harvest in both 2011 and 2012.

The detailed grain weight assessments gave an insight into effects of shading on grain growth at individual zones on the ear and provided further evidence for a lack of grain abortion when post-anthesis photo-assimilation is reduced. It was postulated that if grain abortion were a mechanism for ensuring remaining grains have sufficient assimilate to fill to an adequate size, abortion would be expected to occur at the more distal and basal grain zones where grain size is smaller, and that it may be accompanied by a decline in grain dry matter at these positions as a result of dry matter remobilisation. However there was no evidence of a decline in grain dry matter following 'late' shading in 2011 (main stem only) or 2012 (main stem and two tillers). While the rate of grain growth and final grain weight achieved was reduced by shading, grains at all zones in shaded and unshaded treatments grew steadily. There was also no evidence that growth of grains in central zones was maintained at the expense of those in distal and basal zones after 'late' shading in 2011 or 2012. While there was a consistent zone effect whereby grains in distal and basal zones were lighter than those in central zones, there was no evidence of a greater relative reduction in grain growth at the ear extremities. In fact, significant effects of 'late' shading in 2011 were first detected in central grains. This effect appeared to spread outwards with time and reductions in grain weight due to shading appeared to be equal across zones at harvest. Further, there was no shading x zone interaction effect on main stem ear grain weight at harvest following 'late' shading in 2011 and 2012. Thus, rather than sacrifice the growth of smaller upper and lower grains of the ear to ensure the survival of larger more central grains, partitioning of carbon assimilates appears to occur equally across all grain zones. A similar response has been reported for wheat where growth of grains in different spikelet positions was reduced equally by shading, but growth of florets at different positions within spikelets was altered unequally (Bremner and Rawson, 1978). The results were interpreted in terms of variation in the vascular connections between spikelets and between florets within spikelets and its consequences for the resistance of phloem translocation pathways. However, in the present study interpretation of data at the most extreme upper and lower zones must be approached with caution as large variances were noticeable in these zones throughout the assessments partly due to missing values as not all the sampled ears were long enough to span these distal

zones. Despite this, evidence supports the null hypothesis that a reduction in assimilation capacity post-anthesis does not reduce grain number, at least not via effects on grain number per ear.

The comparison of grain weight distribution histograms for 'late' shading and unshaded treatments in 2011 supports other analyses indicating that the weight of grain at all zones on the ear is reduced more or less equally by late shading. Further, there are no additional grains in the < 5.0 mg classes indicating that there was no increase in the amount of non-grains in the 'late' shading treatment when compared to the unshaded treatment (the estimated mean weight of a non-grain is 2.9 mg). Results are similar to those of Grashoff and dAntuono (1997) when post-anthesis shading was applied to spring barley.

3.5 Conclusion

Calculations of MGW from combine harvested grain samples were not consistently higher than hand harvested estimates of MGW. The hypothesis that the loss of small grains from combine harvesters contributes to the apparent conservation of MGW therefore cannot be accepted (at least for combines set-up correctly as described here). Combine-threshed grain samples can and do collect the same proportion of grain in each weight class as hand-threshed samples except for the < 5.0 mg weight class which is likely to contain some non-grain material.

Given that there was no evidence of abortion of grains within an ear or complete post-anthesis tiller abortion following post-anthesis shading, the hypothesis that a reduction in assimilation capacity post-anthesis reduces grain number in Irish-grown spring barley can be rejected. If neither the loss of grains from combine harvesters nor a post-anthesis grain number adjustment mechanism is responsible for a relatively conserved MGW then this implies that grains consistently fill to a pre-determined storage capacity. A more thorough understanding of the mechanisms responsible for determining grain storage capacity and whether it becomes a yield limiting factor at higher grain numbers is required.

Chapter 4 **Grain storage capacity and the trade-offs between yield components**

4.1 Introduction

Several lines of evidence indicate that the yield of barley and other small grain cereals is largely sink limited. Firstly, grain number is highly associated with yield across environments (Abeledo et al., 2003; Baethgen et al., 1995; Bingham et al., 2007a; Blake et al., 2006; del Moral et al., 2003; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013) even in the high yield potential environment of Ireland (Chapter 2), whilst MGW is relatively conserved (see Chapter 3, section 3.1 and references therein). Secondly, estimates of potential assimilate availability for grain filling in winter barley crops exceeded grain yield across sites and seasons (Bingham et al., 2007a). Moreover, there was a decline in RUE during grain filling at some sites where the source:sink ratio was high; the decline was interpreted as evidence of feedback inhibition of photosynthesis by a limited sink demand for assimilates. Similar evidence of a decline in RUE is presented in Chapter 2. Further evidence is provided in the literature of a surplus of assimilate for grain filling from post-anthesis photosynthesis (Dreccer et al., 1997; Richards, 2000; Serrago et al., 2013; Slafer and Savin, 1994) and pre-anthesis storage reserves (Beed et al., 2007; Fabian et al., 2011; Foulkes et al., 2007; Serrago et al., 2013; Yoshida, 1972). Thirdly, manipulating source:sink ratios during grain filling through degrading or shading treatments have been found to have little effect on grain weight relative to the change in ratio imposed (Beed et al., 2007; Borrás et al., 2004; Estrada-Campuzano et al., 2008; Grashoff and d'Antuono, 1997; Habgood and Uddin, 1983; Jenner, 1979; Serrago et al., 2013; Willey and Holliday, 1971). This has also been demonstrated in Chapter 3. Reynolds et al. (2005) has shown in wheat that if sink demand can be increased, RUE during grain filling can be increased in response to the need for more assimilates resulting in simultaneous increases in final biomass and yield. This was achieved in a field experiment where row lengths of wheat were subject to additional light interception for 15 days prior to anthesis by bending back adjacent row lengths. Collectively these observations suggest that sink limitation of yield, or a co-limitation by source and sink, is the norm rather than source limitation.

A consequence of this is that it might be possible to increase yield by increasing sink capacity as there appears to be assimilate potentially available (or the capacity to generate it) to fill additional grains. However, to realise these yield gains it is necessary to understand what controls sink capacity. Grain sink capacity is a product of the number of grains and their capacity for storing dry matter (potential size) (Evans and Wardlaw, 1996). Evidence from Chapter 3 suggests that grain number is largely determined pre-anthesis. Grain storage capacity (GSC) may be determined prior to anthesis or early post anthesis.

There is evidence in wheat and barley that GSC may be influenced by events shortly before anthesis perhaps through restrictions on carpel weight (Bingham et al., 2007b; Calderini et al., 1999; Calderini and Reynolds, 2000; Calderini et al., 2001; Hasan et al., 2011; Scott et al., 1983). It is possible that hull size and weight are limiting factors to grain growth (Habgood and Uddin, 1983; Scott et al., 1983). Furthermore, GSC can be impacted upon as early as the tillering stage through restrictions on ovary size (Kirby, 1977; Kirby and Jones, 1977). In such scenarios, GSC would be dependent upon the resource distribution among developing florets early in development (Gambín and Borrás, 2010).

Post-fertilisation grain development can be considered in three phases: (1) a period of cell division during which most of the cells of the endosperm are formed (Kirby and Appleyard, 1984); (2) a period of rapid grain filling when the grain accumulates dry matter (Slafer et al., 2009); (3) a ripening period of grain dehydration prior to harvest. The initial endosperm cell division period in barley can continue for up to 30 days post-anthesis (Cochrane and Duffus, 1981, 1983; Evers, 1970; Kvaale and Olsen, 1986; Nicolas et al., 1985; Radley, 1978) however the division of starchy type endosperm cells, which contribute most to grain weight, ceases approximately 14-23 days after anthesis (Cochrane and Duffus, 1981, 1983; Kvaale and Olsen, 1986; Olsen and Krekling, 1980). Positive correlations have been shown between the number of endosperm cells and MGW in wheat (Brocklehurst, 1977; Gleadow et al., 1982; Hasan et al., 2011; Nicolas et al., 1985) and barley (Cochrane and Duffus, 1983). In UK grown barley, Bingham et al. (2007b) also found a significant positive linear relationship between MGW and the amount of photosynthetically active

radiation (PAR) intercepted per unit grain number between ear emergence and the start of rapid grain filling – the period during which you would expect the basis of GSC to be set. Furthermore, shading and drought treatments applied to wheat plants in controlled environments for the approximate endosperm cell division period resulted in reductions in grain weight at harvest ripeness (Fabian et al., 2011; Jenner, 1979; Nicolas et al., 1985) with effects attributed to a reduced endosperm cell number rather than a consequence of depleted levels of assimilate per grain for subsequent grain dry matter accumulation (Jenner, 1979).

Endosperm cell size and starch granule size increase towards the center of the endosperm (Evers and Millar, 2002). It is unclear whether this is due to central cells (first formed) having had longer to produce storage products, or whether it is simply an inherent product of varying cell differentiation dependent on location in the endosperm (Evers and Millar, 2002). It is also unclear when endosperm cell size is determined. While endosperm cell size is a component of GSC it is unlikely to have as strong an influence on GSC and grain size as endosperm cell number (Cochrane and Duffus, 1983; Dunstone and Evans, 1974).

Evidence from Chapter 2 shows that a barley canopy with a green area index (GAI) of 5-6 intercepts greater than 93% of incident solar radiation. As the relationship between canopy GAI and light interception is non-linear, it is conceivable that increasing grain number in crops whose GAI is already greater than 5-6 will lead to reductions in the amount of light intercepted per unit grain number. This, in turn, could restrict endosperm cell division and MGW, thus limiting the possible yield benefits of increasing grain number. However, the endosperm cell division phase is a period when the demand for assimilate by the grain is relatively low and when stem water soluble storage reserves are being deposited (Gallagher et al., 1975). If grain development takes priority over storage deposition for the available assimilates, grain development would be expected to be relatively insensitive to variation in light interception. The following hypothesis is proposed:

- MGW is insensitive to variations in incident light during early grain development because grain soluble sugar concentrations are maintained at the expense of stem storage reserves.

Although the available evidence points to a sink limitation of yield in barley, or co-limitation by source and sink as the norm, evidence from Chapter 3 and other authors (Grashoff and dAntuono, 1997; Serrago et al., 2013; Willey and Holliday, 1971) has shown that substantial reductions in incident light during rapid grain filling imposed through shading treatments can lead to reductions in MGW. This suggests that crops may shift from a position of sink limitation to source limitation of grain filling when reductions in assimilate availability are extreme enough. Moreover, if grain number and GSC are both determined at the same time prior to anthesis there are likely to be trade-offs between grain number and grain weight (as determined by GSC) which could limit the possible yield benefits of increasing grain numbers. Whilst there is often no correlation between grain number and MGW across sites and seasons (Chapter 2), within a given environment negative relationships have been reported when grain numbers were varied experimentally (Grashoff and dAntuono, 1997; Jenkyn et al., 1992).

A second objective of experiments conducted here was to vary grain number per unit area by modifying seed rates in order to investigate the possible trade-offs between yield components.

4.2 Materials and Methods

4.2.1 Modification of photosynthetic assimilation capacity ‘early’ post-anthesis

4.2.1.1 *Experimental design, treatments and husbandry*

‘Early’ shading treatments were applied to crops of a high yield potential two-row spring barley variety (*Hordeum vulgare* L. cv. Quench) at Oakpark, Co Carlow (CW) in 2012 and 2013 for a period of 14 days immediately after anthesis. Site and crop management details are described in Chapter 2, section 2.2.1. A standard seed rate of 330 seeds m⁻² was used in 2012 while in 2013 shading treatments were applied to low, standard and high seed rates of 80, 320 and 1280 seeds m⁻² respectively. Plot size in 2012 was 4 m x 24 m and 2.5 m x 12 m in 2013. Shades were erected over sub-plots of 2 m x 3 m in 2012 and over an increased area of 2.5 m x 6 m in 2013 to allow for a greater amount of destructive sampling in that season. The shading material used was an open weave polystyrene shade-netting (Tildenet Ltd., Bristol, UK) which gave a 59% PAR reduction in 2012 and a 72 % reduction in 2013 due to the use of a slightly closer weave shade netting material in that season. Shades were erected on a frame of fencing posts and rope at a height of 1.1 m above ground level (Figure 3.1). Anthesis was judged to occur when half of the ear had emerged on half of all shoots which corresponded to GS 55 on the Zadoks scale (Zadoks et al., 1974). A randomized block design was used in 2012 with four replicates while a split-plot design with four replicates was employed in 2013 with seed rate as the whole-plot and shading as the sub-plot.

An additional row-opening experiment was carried out at CW in 2012 to further test the hypothesis that MGW in barley crops of high grain number is insensitive to variations in incident light during early grain development. Solar radiation available to treated row lengths of spring barley cv. Quench was increased by gently bending back the adjacent crop using a system of upright stakes and horizontal bamboo canes (Figure 4.1). This opening-up treatment, was applied to 4 m long row lengths of crop in plots of both 330 seeds m⁻² and 660 seed m⁻² for the same developmental period as

the ‘early’ shading treatment described above (i.e. for 14 days commencing at GS 55). After the 14 days the canes were removed and the rows allowed to close-up again. Plot size was 4 m x 24 m and untreated and treated row lengths were located within the same plot in a split-plot design with four replicates with seed rate as the whole-plot and opening-up treatment as the sub-plot.



Figure 4.1. An opened-up treatment applied to a 330 seeds m⁻² plot at CW in 2012.

4.2.1.2 In-field assessments and destructive sampling

Plant population counts were carried out by counting the number of plants both sides of a 0.5 m marker at five locations per plot and converted to area based measurements using the row width. Soil moisture, crop height, incident PAR and leaning and lodging were monitored in shaded ‘early’ and unshaded treatments as

described in Chapter 3, section 3.2.2.2. In the row-opening experiment, the amount of PAR reaching the base of the canopy was measured using the hand-held probe of a Sunscan Canopy Analysis System (Delta T Devices, Cambridge, UK) placed along the ground parallel to the opened-up row length and compared to readings from control (unopened) row lengths of crop.

At harvest ripeness in the shading experiments, a quadrat sample size of 6 x 1 m undisturbed adjacent row lengths of above ground crop material (equating to 0.72 m²) was removed from each treatment area and air-dried prior to processing. A 40% sub-sample (by shoot number) was obtained for above ground dry matter determination and a further 20% was separated into ears and straw. At harvest ripeness in the row-opening experiment, a 1 m row length of above-ground crop material was removed from each treatment area and air-dried prior to processing. The number of ears in the entire sample was counted before separation into ear and straw portions for dry weight determination. All samples were oven dried at 70 °C for 48 hours (or to a constant mass) then processed as described in Chapter 3, section 3.2.2.3 to obtain yield, yield component and harvest biomass data.

Additional 1 m row lengths of crop were removed from each shading treatment area in 2013 at the beginning and end of the shading treatment (GS 55 and GS 55 + 14 days) and again at physiological maturity (GS 87) to track the progress of post-anthesis dry matter partitioning in shaded and unshaded treatments. Whole plants (including roots) were sampled and stored in sealed bags in order to prevent drying out and if the subsequent growth analysis in the laboratory was delayed, samples were stored in a cold room at 4-6 °C. The total number of plants in each sample was counted before a 50% sub-sample (by plant number) was separated into ears, straw and leaf portions after the number of fertile shoots/ears had been counted. Samples were dried at 70 °C for 48 hours (or to a constant mass) and the dry weight of each portion was then determined and expressed on a per shoot basis.

There was at least 0.5 m distance between adjacent destructive sampling areas which were at least 0.5 m from the ends and edges of plots/treatments. Tram lines and drill overlaps were also avoided with the aim of selecting sample areas that were representative of the plot.

4.2.1.3 Determination of stem, grain and chaff soluble carbohydrate concentration

Ten randomly selected viable shoots per treatment were sampled from the 2013 shading experiment at GS 55, GS 55 + 14 days, GS 55 + 21 days and GS 87 to measure soluble sugar concentrations. The shoots were immediately separated into ears and stems and the fresh weight of each portion was recorded before drying as described by (Bingham et al., 2007a). Ears were then threshed between two pieces of foam board and sieved over a mechanically operated 1 mm slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany) to separate into chaff and grain portions (Figure 2.5). Grain portions included the lemma and palea but not the awn. Where chaff material remained in the grain portion on top of the sieve it was removed with tweezers and added to the chaff portion. For the GS 55 samples when no grain was present, ears were lightly threshed by hand to separate spikelets from the rachis and other chaff. Samples were then sieved as above and awns removed to separate 'grain' samples that contained only the lemma, palea and developing embryo parts of spikelets. Following determination of dry weight for each portion, tissue was milled to a fine powder in a cross beater type mill (Glen Creston, London, UK) and sub-samples of approximately 30 mg were taken and weighed to the nearest 1.0 mg. Stem sub-samples were extracted sequentially in 80% v/v ethanol:water, 50% ethanol:water and then in deionised water and the extracts pooled for analysis. The sequential extraction was to remove low molecular weight sugars and fructans of low and high degree of polymerisation. Grain and chaff sub-samples from the first three sampling occasions only were extracted in 80% v/v ethanol:water to remove just low molecular weight sugars (mostly hexoses). The extracts were evaporated to dryness in a centrifugal evaporator (Thermo Fischer Scientific Inc., Waltham, USA) set for 90 minutes at 70°C with pulse ventilation. Extracts were then re-suspended in a known volume of deionised water and the soluble sugar concentration determined colourimetrically using the phenol-sulphuric acid method of (Bingham et al., 2012; DuBois et al., 1956).

4.2.2 Further seed rate treatments

4.2.2.1 Experimental design and site characterisation

To investigate the potential trade-offs between MGW and grain number and to inform hypotheses on how increases in grain number per unit area can be achieved field experiments were established at Oakpark, Carlow (CW) and Kildalton, Kilkenny (KK) in 2013. Spring barley cv. Quench was sown at six seed rate treatments of 40, 80, 160, 320, 640 and 1280 seeds m⁻² on the 20th of March at CW and on the 23rd of April at KK. At each site the experiments were laid out in one bank of plots with four fully randomised blocks. Plots were 2 m wide and 24 m long with half the length of the plot designated for combine harvesting and the other half for destructive sampling. Sites fitted in with a standard barley rotational position and were managed for high yield potential as described in Chapter 2, section 2.2.1. Site details for CW already given in Chapter 2 are repeated here in Table 4.1 along with site details for KK. Soil texture was identified using the method described by Tennyson et al. (2006).

Table 4.1. Latitude/longitude, altitude, and soil texture for the Oakpark, Co. Carlow (CW) and Kildalton, Co. Kilkenny (KK) experimental sites.

Site	Latitude/Longitude	Altitude (m)	Soil texture
CW	52° 51' N, 6° 54' W	57	loam with moderate moisture holding capacity
KK	52° 20' N, 7° 18' W	16	silt loam with moderate moisture holding capacity

4.2.2.2 In-field assessments and destructive sampling

Plant population counts were carried out shortly after emergence by counting the number of plants in a 0.5 m x 0.5 m quadrat at five random locations per plot. Crop height, leaning and lodging at harvest and canopy senescence post-anthesis were measured in each plot as described in Chapter 3, section 3.2.2.2.

At harvest ripeness, a quadrat sample size of 6 x 1 m undisturbed adjacent row lengths of above ground crop material (equating to 0.72 m²) was removed from each treatment area and air-dried prior to processing. A 20% sub-sample (by shoot number) was obtained for above ground dry matter determination and a further 10% was separated into ears and straw. Samples were then processed as described in Chapter 3, section 3.2.2.3 to obtain yield, yield component and harvest biomass data. On the same day in KK and one week later in CW the portion of the plot designated for combine harvesting was harvested with a Sampo 2010 (Sampe-Rosenlew Ltd., Finland) plot harvester. Grain from each plot was weighed by the harvester independently and a sample was taken for moisture content and MGW determination. Grain yield and its components were then calculated as described for the pre-harvest quadrat sample. Combine threshing and separation apparatus were set up with the objective of achieving a clean sample while losing as little grain as possible.

4.2.3 Statistical analysis

Plant population, harvest yield, yield component, biomass and other data obtained from each experiment were analysed statistically for main treatment effects (and interaction effects where applicable) using ANOVA in GenStat (14th Edition, VSN International Ltd., Hemel Hempstead, UK), with the relevant treatment structures described. Data were checked for normality of residuals and homoscedasticity prior to analysis. Following ANOVA, relevant means for treatments were compared using the standard error of the difference (S.E.D.) between means, on the residual degrees of freedom (d.f.) from the ANOVA, thus invoking the least significant difference (L.S.D.) at the P = 0.05 level of significance.

Dry matter partitioning and soluble sugar concentration data for individual plant portions in the 2013 shading experiment were analysed using repeated measures ANOVA in GenStat where 'time' (sampling occasion) was included as a factor to account for the correlation between data on successive sampling occasions.

Relationships between variables at the CW and KK seed rate experiments were assumed to be non-linear if the addition of the quadratic term to a linear regression in GenStat proved to be a significant improvement ($P < 0.05$). If curvature was detected relationships were fitted with either a second order polynomial (quadratic) regression or a split-line regression.

4.3 Results

4.3.1 Responses to shading ‘early’ post-anthesis

Climate and site information for the CW site in 2012 and 2013 are given in Chapter 2, section 2.3.1.

4.3.1.1 *Crop development, micro-climate, leaning and lodging*

Percentage plant establishment in 2012 from the seed rate of 330 seeds m⁻² was 96 %. Establishment for the three seed rates in 2013 was as follows: 80 seeds m⁻²: 49 %; 320 seeds m⁻²: 57 %; 1280 seeds m⁻²: 38%. Seed rate in 2013 had a significant positive effect on plant number m⁻² ($P < 0.001$). There was also an observed developmental effect of seed rate – the high seed rate reached anthesis (GS 55) four days prior to the standard seed rate and the standard seed rate reached anthesis four days prior to the low seed rate. The timing of growth stage dependent crop sampling was adjusted accordingly for each seed rate.

Shaded environments and the effects of shading on crop development in 2012 are described in more detail in Chapter 3, section 3.3.2.1, therefore only additional details pertinent to the 2013 experimentation are presented here.

Percentage soil moisture (w/w) in the 0 – 30 cm profile at the end of the 14 day shading period in 2013 was 13% for the shading treatment and was significantly higher than the unshaded control value of 11 % ($P = 0.010$); there was no difference in 2012. Similar to other seasons, there was no significant effect of shading on crop height in 2013. The pattern of senescence for ‘early’ shaded plots was similar to unshaded controls. PAR reduction due to shading was consistent at each measurement. There was a significant effect of ‘early’ shading on leaning (stems 0-5° from the horizontal) at harvest ripeness in 2013, but not on lodging or brackling - 5% of all stems were leaning compared to 2% in the unshaded control (based on a whole plot visual assessment). However this will have had a negligible effect on PAR interception and RUE.

4.3.1.2 Yield and yield components

There were large differences in the control values of yield and yield components between seasons. Unshaded yield in 2012 of was 1.91 t ha⁻¹ higher than the unshaded yield for the equivalent seed rate (320 seeds m⁻²) in 2013 (Table 4.2). Harvest grain number m⁻² was also higher in 2012 however MGW was 22 % lower in 2012 than in 2013.

In 2012 ‘early’ shading had no significant effect on yield, grain number or MGW at harvest (Table 4.2). Straw biomass at harvest was significantly reduced by shading ($P = 0.029$), however, there were no other significant effects on harvest variables in 2012. In 2013, where three seed rates were included to alter the number of grains relative to canopy light interception at anthesis, there was also no significant effect of ‘early’ shading on MGW. However there were significant reductions in total biomass ($P = 0.010$) and ear biomass at harvest ($P = 0.018$) in response to ‘early’ shading along with a significant reduction in biomass accumulated during the shading period - a 66% reduction in accumulated biomass in response to a 72% reduction in PAR. Also, an 8% reduction in grain number ($P = 0.046$) in response to ‘early’ shading in 2013 resulted in 7% reduction in yield ($P = 0.036$). However reductions in the sub-components of grain number – 6% for ear number per m² and 3% for grain number per ear were not statistically significant ($P > 0.05$).

In 2013 the lowest seed rate had a significantly higher MGW at harvest ($P = 0.007$) than the standard and high seed rates between which there was no significant difference (Table 4.2). There were significant negative effects of increasing seed rate on grain number per ear ($P < 0.001$), harvest index ($P = 0.014$) and ear number per plant ($P < 0.001$) at harvest. There were also significant positive effects of increasing seed rate on yield ($P = 0.004$), grain number m⁻² ($P = 0.004$), ear number m⁻² ($P < 0.001$), total biomass ($P = 0.006$), ear biomass ($P = 0.006$), and straw biomass ($P = 0.003$) at harvest. However there was no significant seed rate x shading interaction for any harvest variable in 2013.

Table 4.2. Mean values of yield, yield components and other harvest variables for unshaded and 'early' shaded treatments in 2012. Values for main effect means of shading and seed rate treatments in 2013 are also given. *P* values and L.S.D. 5% (least significant difference at $P = 0.05$) values are for shading, seed rate and shading \times seed rate effects following ANOVA; DM = 100% dry matter; yield and MGW expressed at 85% dry matter.

SHADING	2012				2013											
	Means		Shading effects		Means		Shading effects		Means			Seed rate effects			Seed rate \times sh. effects	
	Unshade d	Shaded 'early'	<i>P</i>	<i>d.f.</i>	Unshade d	Shaded 'early'	<i>P</i>	<i>d.f.</i>	80 seeds m ⁻²	320 seeds m ⁻²	1280 seeds m ⁻²	<i>P</i>	L.S.D . 5%	<i>d.f.</i>	<i>P</i>	<i>d.f.</i>
Yield (t ha ⁻¹)	7.98	7.34	0.100	3	6.24	5.78	0.036	9	5.00	6.07	6.97	0.004	0.85	6	0.068	9
Grain no. m ⁻²	20335	18361	0.265	3	12475	11443	0.046	9	9308	12283	14287	0.004	2130	6	0.139	9
MGW (mg)	39.33	40.05	0.674	3	50.40	51.06	0.323	9	53.83	49.55	48.81	0.007	2.61	6	0.874	9
Ear no. m ⁻²	997	935	0.297	3	909	851	0.345	9	454	818	1368	<0.001	200	6	0.247	9
Grain no. ear ⁻¹	20.39	19.69	0.487	3	15.66	15.21	0.372	9	20.53	15.23	10.55	<0.001	1.60	6	0.661	9
Harvest index	50.87	53.15	0.103	3	60.16	60.28	0.832	9	61.30	60.81	58.53	0.014	1.67	6	0.224	9
Ear no. plant ⁻¹	3.1	2.9	0.297	3	7.4	6.0	0.241	9	12.3	4.9	2.9	<0.001	2.8	6	0.102	9
Total biomass (t ha ⁻¹ DM)	13.35	11.98	0.181	3	9.21	8.34	0.010	9	7.19	8.67	10.46	0.006	1.53	6	0.099	9
Ear biomass (t ha ⁻¹ DM)	8.18	7.39	0.060	3	6.36	5.83	0.018	9	5.09	6.16	7.04	0.006	0.92	6	0.133	9
Straw (incl. leaf) biomass (t ha ⁻¹ DM)	5.59	4.85	0.029	3	2.74	2.55	0.090	9	2.02	2.56	3.36	0.003	0.57	6	0.545	9
Biomass acc. during shading (t ha ⁻¹ DM)	4.13	-	-	-	3.98	1.37	0.013	9	2.61	3.65	1.75	0.096	ns	6	0.952	9

4.3.1.3 Dry matter partitioning for the 2013 ‘early’ shading experiment

In 2013, unshaded ear biomass increased by 0.19 g per shoot during the first two weeks after anthesis after which it increased at a faster rate up to physiological maturity (GS 87); there was little change between GS 87 and harvest ripeness (Figure 4.2). Repeated measures ANOVA showed no significant effect of shading ($P = 0.180$) nor a time \times shading interaction ($P = 0.549$) for ear biomass meaning that there was no significant difference in ear biomass growth between shaded ‘early’ and unshaded treatments at any sampling occasion (Table 4.3; Figure 4.2). Unshaded stem biomass increased by 0.16 g per shoot during the first two weeks immediately post-anthesis then declined through to GS 87 and again from GS 87 to harvest ripeness. There was a significant time \times shading interaction effect for stem biomass where stem biomass in the ‘early’ shading treatment was 21% lower than the unshaded control ($P < 0.05$, L.S.D. = 0.04) at the end of the shading period (GS 55 + 14 days). Thereafter there was no difference between treatments as the stem DW of unshaded controls declined to values similar to those of previously shaded plants. Leaf biomass in the unshaded control remained stable during the two weeks immediately post-anthesis then declined by 0.05 g per shoot through to harvest ripeness. As with ear biomass, there was no significant difference in leaf biomass growth between shaded and unshaded treatments at any sampling occasion (time \times shading interaction $P = 0.430$).

There was a significant negative effect of increasing seed rate on ear, stem and leaf biomass per shoot (P always < 0.001) where values were lower at higher seed rates. There was also a significant time \times seed rate interaction effect for each dry matter portion where the magnitude of the differences between seed rates increased with time for ears ($P < 0.001$) and decreased from GS 55 + 14 days onwards for stem ($P < 0.001$) and leaf ($P = 0.002$) portions. However, there was no significant seed rate \times shading or time \times seed rate \times shading interaction effect implying that the effects of shading on a particular variable were comparable at each seed rate.

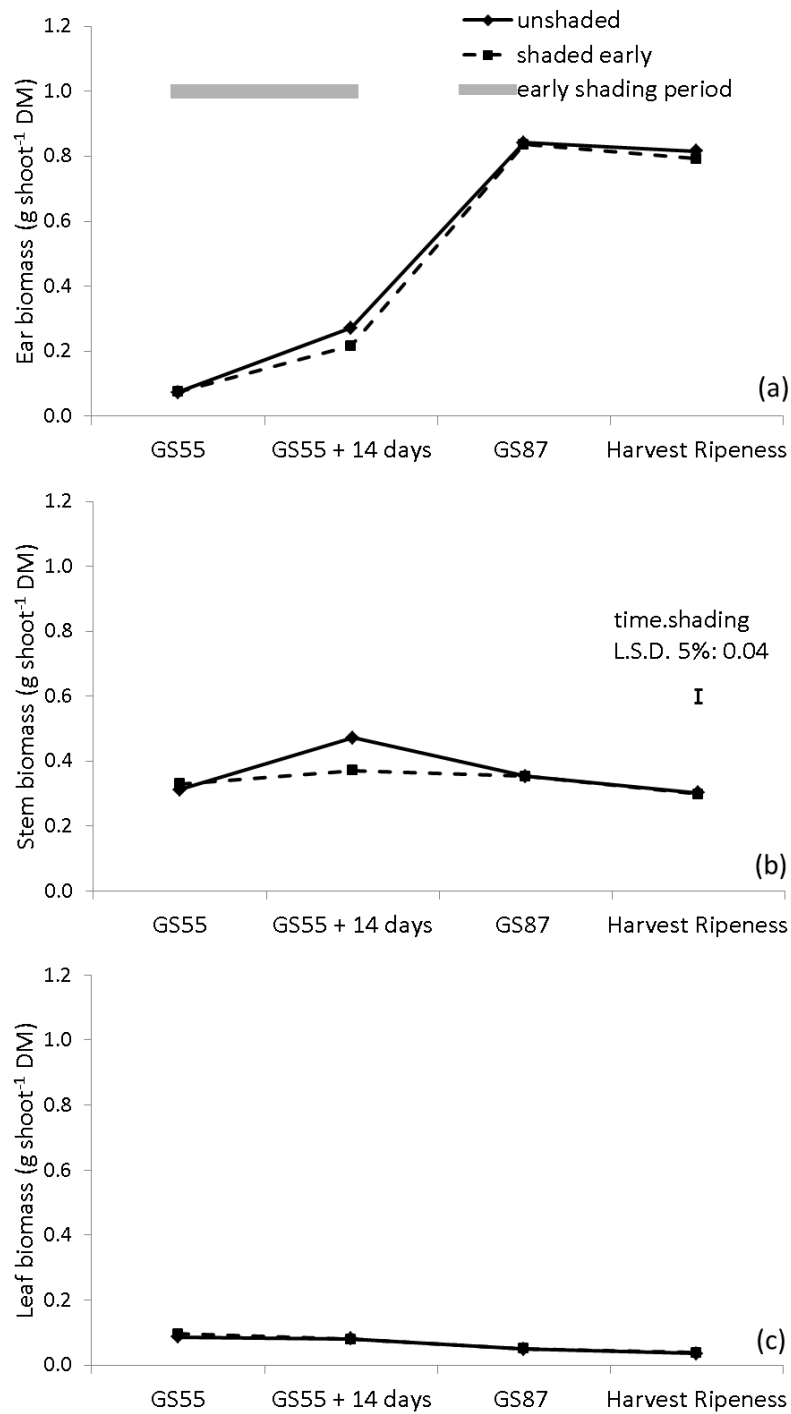


Figure 4.2. Unshaded and shaded 'early' biomass shoot⁻¹ for (a) ear, (b) stem and (c) leaf portions at a range of post-anthesis developmental stages in 2013. Values are the means of four replicates and three seed rates. The error bar is the L.S.D. 5% (least significant difference at $P = 0.05$) for the time of sampling \times shading interaction effect following repeated measures ANOVA. DM = 100% dry matter. The duration of the 'early' shading period is shown as a shaded area in (a).

Table 4.3. Significance of effects of seed rate, shading and sampling time on ear, stem and leaf biomass shoot¹ for data collected at a range of post-anthesis developmental stages in 2013 after repeated measures ANOVA; interaction effects are also given; L.S.D. 5% = least significant difference at $P = 0.05$.

<i>Effects</i>	<i>P</i>	<i>L.S.D. 5%</i>	<i>d.f.</i>
<i>Ear biomass shoot¹</i>			
<i>Seed rate</i>	<i><.001</i>	<i>0.038</i>	<i>15</i>
<i>Shading</i>	<i>0.180</i>	<i>ns</i>	<i>15</i>
<i>Seed rate.Shading</i>	<i>0.813</i>	<i>ns</i>	<i>15</i>
<i>Time</i>	<i><.001</i>	<i>0.053</i>	<i>30</i>
<i>Time.Seed rate</i>	<i><.001</i>	<i>0.088</i>	<i>42</i>
<i>Time.Shading</i>	<i>0.549</i>	<i>ns</i>	<i>42</i>
<i>Time.Seed rate.Shading</i>	<i>0.690</i>	<i>ns</i>	<i>42</i>
<i>Stem biomass shoot¹</i>			
<i>Seed rate</i>	<i><.001</i>	<i>0.027</i>	<i>15</i>
<i>Shading</i>	<i>0.052</i>	<i>0.022</i>	<i>15</i>
<i>Seed rate.Shading</i>	<i>0.726</i>	<i>ns</i>	<i>15</i>
<i>Time</i>	<i><.001</i>	<i>0.030</i>	<i>45</i>
<i>Time.Seed rate</i>	<i><.001</i>	<i>0.052</i>	<i>60</i>
<i>Time.Shading</i>	<i>0.001</i>	<i>0.042</i>	<i>60</i>
<i>Time.Seed rate.Shading</i>	<i>0.672</i>	<i>ns</i>	<i>60</i>
<i>Leaf biomass shoot¹</i>			
<i>Seed rate</i>	<i><.001</i>	<i>0.009</i>	<i>15</i>
<i>Shading</i>	<i>0.382</i>	<i>ns</i>	<i>15</i>
<i>Seed rate.Shading</i>	<i>0.834</i>	<i>ns</i>	<i>15</i>
<i>Time</i>	<i><.001</i>	<i>0.008</i>	<i>24</i>
<i>Time.Seed rate</i>	<i>0.002</i>	<i>0.015</i>	<i>39</i>
<i>Time.Shading</i>	<i>0.430</i>	<i>ns</i>	<i>39</i>
<i>Time.Seed rate.Shading</i>	<i>0.277</i>	<i>ns</i>	<i>39</i>

4.3.1.4 Sugar concentrations for the 2013 'early' shading experiment

In 2013, the ethanol soluble carbohydrate (ESC) concentration in the grain of the unshaded crop increased from 4.8% to 7.6% (w/w) during the two weeks immediately post-anthesis after which it declined (Figure 4.3 (a)). The same was true for the ethanol and water soluble carbohydrate (EWSC) concentration in the stem where an increase from 13.4% to 15.5% was recorded during the first two weeks post-anthesis before concentrations began to decline (Figure 4.3 (c)). Unshaded chaff ESC concentration decreased slightly during this two week period before returning to anthesis levels in the following week (Figure 4.3 (b)).

There was no significant difference between shaded and unshaded treatments for grain ESC concentration ($P = 0.339$) at any sampling occasion (Table 4.4; Figure 4.3). However the same was not true for chaff ESC concentration which, at the end of the treatment period (GS 55 + 14 days), was significantly reduced by shading compared to the unshaded control ($P < 0.05$, L.S.D. = 0.51) after which it returned to unshaded levels. Concentrations of low molecular weight sugars plus fructans (EWSC) in the stems of the shaded crop were also significantly reduced at the end of the treatment period ($P < 0.05$, L.S.D. = 1.14). Concentrations remained significantly lower than controls a week after the shading was removed, but the difference was reduced considerably (from a difference of 5.9% DW to 2.2 % DW). Eventually stem EWSC concentrations fell to similar levels in both previously shaded and control crops by physiological maturity (GS87).

There was no significant seed rate x shading interaction effect for any crop portion indicating that shading influenced soluble carbohydrate concentrations in the same way irrespective of seed rate.

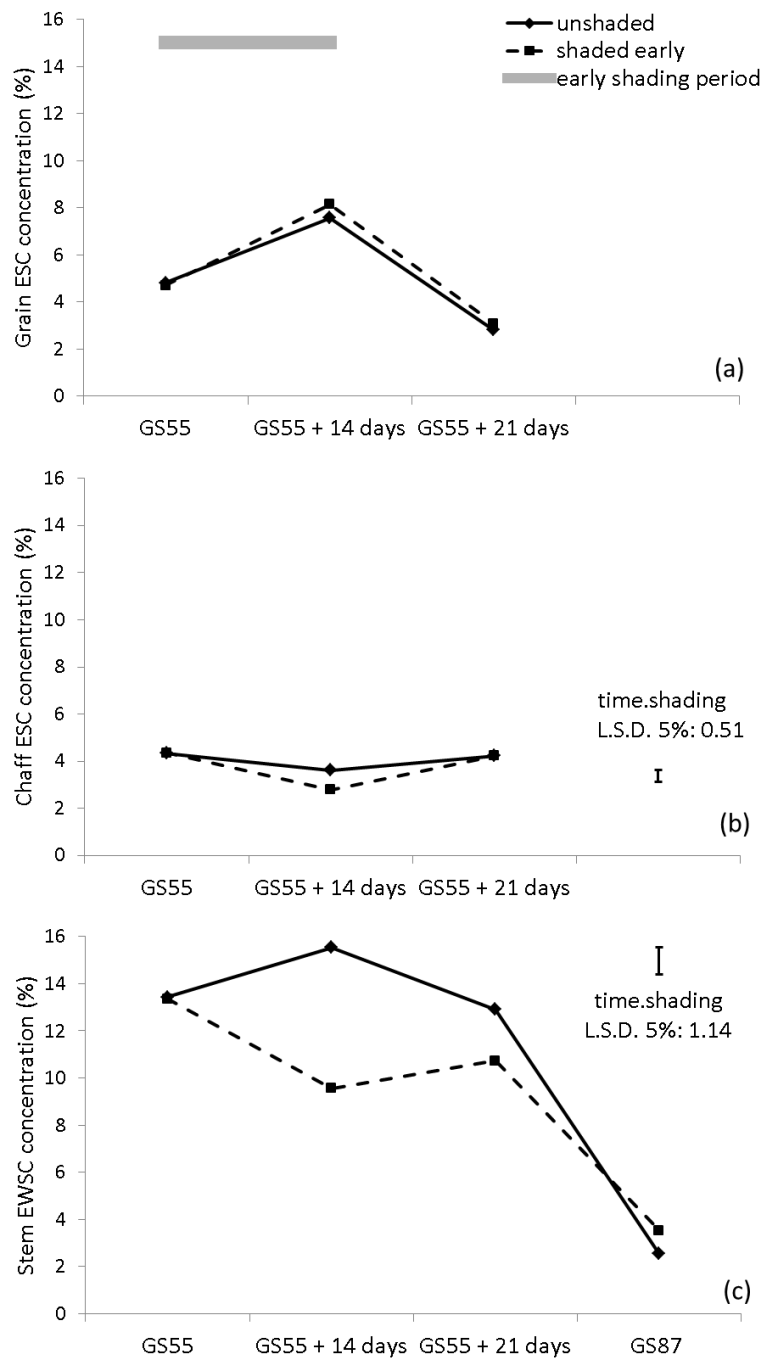


Figure 4.3. Unshaded and shaded 'early' ethanol soluble carbohydrate (ESC) concentrations for grain (a) and chaff (b) along with ethanol and water soluble carbohydrate concentration (EWSC) for stem (c) at a range of post-anthesis developmental stages in 2013. Values are the means of four replicates and three seed rates. The error bar is the L.S.D. 5% (least significant difference at $P = 0.05$) for the time of sampling \times shading interaction effect following repeated measures ANOVA. The duration of the 'early' shading period is shown as a shaded area in (a).

Table 4.4. Significance of effects of seed rate, shading and sampling time on grain and chaff ethanol soluble carbohydrate (ESC) concentrations and stem ethanol and water soluble carbohydrate (EWSC) concentration after repeated measures on data collected at a range of post-anthesis developmental stages in 2013; interaction effects are also given; L.S.D. 5% = least significant difference at $P = 0.05$.

Effects	P	L.S.D. 5%	d.f.
Grain ESC %			
Seed rate	0.078	ns	15
Shading	0.412	ns	15
Seed rate.Shading	0.447	ns	15
Time	<.001	0.53	22
Time.Seed rate	0.002	1.07	35
Time.Shading	0.339	ns	35
Time.Seed rate.Shading	0.294	ns	35
Chaff ESC %			
Seed rate	0.022	0.35	15
Shading	0.058	0.28	15
Seed rate.Shading	0.177	ns	15
Time	<.001	0.38	35
Time.Seed rate	0.004	0.63	50
Time.Shading	0.047	0.51	50
Time.Seed rate.Shading	0.372	ns	50
Stem EWSC %			
Seed rate	0.006	1.05	15
Shading	<.001	0.86	15
Seed rate.Shading	0.256	ns	15
Time	<.001	0.64	46
Time.Seed rate	0.009	1.40	42
Time.Shading	<.001	1.14	42
Time.Seed rate.Shading	0.006	1.98	42

At the end of the ‘early’ shading period, relative (%) reductions in soluble carbohydrate concentrations were greater than the relative reductions in biomass shoot⁻¹ for both chaff and stem portions (Table 4.5). When concentrations were expressed per unit tissue water for stem and whole ear portions, a significant

reduction in the stem EWSC of 37.1% ($P < 0.001$) was observed in conjunction with a small increase in the ear ESC of 9.6% (ns, $P = 0.147$).

Table 4.5. Relative (%) chaff ethanol soluble carbohydrate (ESC) and stem ethanol and water soluble carbohydrate (EWSC) concentration reductions due to ‘early’ shading at the end of the treatment period (GS 55 + 14 days). Corresponding relative (%) biomass shoot⁻¹ reductions are also given.

At GS 55 + 14	Reduction in ESC/EWSC concentration	Reduction in biomass shoot ⁻¹
Chaff	-23%	-15%
Stem	-39%	-21%

4.3.2 Responses to row-opening ‘early’ post-anthesis

Climate and site information for the CW site in 2012 is given in Chapter 2, section 2.3.1.

4.3.2.1 Crop development, micro-climate, leaning and lodging

The standard seed rate (330 seeds m⁻²) plant population of 317 m⁻² was significantly lower than the high seed rate (660 seeds m⁻²) plant population of 601 m⁻² ($P = 0.002$). There was an observed developmental effect of seed rate where the higher seed rate reached anthesis (GS 55) three days prior to the standard seed rate. The amount of PAR reaching the base of the canopy in opened row-lengths was 2.8 times higher than in unopened row-lengths. There was no observed treatment effect on crop height or canopy senescence. Following removal of the treatment the adjacent row lengths that had been held back leaned in towards the treated row for a period of 2 – 3 days over which they returned to an upright position similar to the treated row. There was no observed leaning or lodging after this point.

4.3.2.2 Yield and yield components

The high seed rate treatment had no significant effect on MGW in this experiment ($P = 0.118$; Table 4.6). Increasing seed rate did significantly reduce grain number per ear ($P = 0.008$) and ear number per plant ($P = 0.004$) but did not significantly increase ear number m^{-2} , grain number m^{-2} , or yield.

There was no significant effect of opening-up rows on yield, grain number m^{-2} or MGW (Table 4.6). While there was also no significant effect of opening-up on ear number m^{-2} , there was on grain number per ear ($P = 0.004$) where there were 0.91 fewer grains per ear in the opened row-lengths than in the unopened. There was also a significant interaction between row-opening and seed rate on grain number per ear ($P = 0.028$) where the relative reduction due to opening-up was greater for the standard seed rate (7%) than the higher seed rate (2%).

Table 4.6. Main effect mean values of yield, yield components and other harvest variables for row-opening and seed rate treatments at CW in 2012. *P* values and L.S.D. 5% (least significant difference at $P = 0.05$) values are for opening-up, seed rate and seed rate \times opening-up effects following ANOVA; ns = non-significant; DM = 100% dry matter; yield and MGW expressed at 85% DM

ROW- OPENIN G 2012											
	Means		Openin g-up effects		Means		Seed rate effects		Seed rate \times opening-up effects		
	Unop ened	Opened 'early'	<i>P</i>	<i>d.</i> <i>f.</i>	330 seeds m ⁻²	660 seeds m ⁻²	<i>P</i>	<i>d.f.</i>	<i>P</i>	L.S.D . 5%	<i>d.f.</i>
Yield (t ha ⁻¹)	7.38	6.96	0.3 08	6	7.40	6.95	0.27 4	3	0.472	ns	9
Grain no. m ⁻²	1726 4	16406	0.3 91	6	17165	16505	0.50 3	3	0.327	ns	8
MGW (mg)	42.76	42.52	0.6 73	6	43.13	42.15	0.11 8	3	0.251	ns	7
Ear no. m ⁻²	952	955	0.9 53	6	887	1019	0.13 8	3	0.602	ns	7
Grain no. ear ⁻¹	18.23	17.32	0.0 04	6	19.36	16.18	0.00 8	3	0.028	1.48	4
Harvest Index	51.59	50.47	0.2 27	6	50.57	51.49	0.19 1	3	0.404	ns	9
Ear no. plant ⁻¹	2.1	2.1	0.9 28	6	2.6	1.6	0.00 4	3	0.624	ns	8
Total biomass (t ha ⁻¹ DM)	12.58	12.11	0.5 78	6	12.83	11.86	0.25 4	3	0.764	ns	9
Ear biomass (t ha ⁻¹ DM)	7.51	7.11	0.3 45	6	7.56	7.06	0.26 1	3	0.497	ns	8
Straw+leaf biomass (t ha ⁻¹ DM)	5.07	4.99	0.8 65	6	5.27	4.79	0.26 1	3	0.952	ns	9

4.3.3 Further seed rate treatments

4.3.3.1 Crop development, micro-climate, leaning and lodging

Climate and site information for the CW site in 2013 are given in Chapter 2, section 2.3.1. Similar information was unavailable for the KK site in 2013 where the nearest national met station was located 60 km east at Johnstown Castle, Co. Wexford. Data from this station in 2013 are presented as 'WX 2013' in Chapter 2, section 2.3.1.

Increasing seed rate had a significant positive effect on plant number m^{-2} at both CW ($P = < 0.001$) and KK ($P = < 0.001$; Figure 4.4). There was significant curvature in the relationship at CW only ($P = 0.001$) therefore these data were fitted with a second order polynomial regression. Percentage plant establishment was greater at KK than CW across seed rate treatments with the difference accentuated at the higher seed rates (Figure 4.4; Table 4.7). Standard deviations of the plant population measurements were relatively high when compared to means, particularly at lower seed rates and particularly at CW where establishment was poorer and visibly more variable within a given plot (Table 4.7).

There was no significant effect of seed rate on crop height at CW ($P = 0.160$) however there was at KK ($P = 0.017$) where the 1280 seeds m^{-2} treatment was significantly shorter than the other seed rates between which there was no difference. There was an observed developmental effect of seed rate where there was a 1 – 3 day time lag between the date of anthesis (GS 55) for each seed rate; higher seed rates reached anthesis earlier. A delay in leaf senescence was observed in the two lowest seed rates at CW with little difference between the other seed rates. A similar trend was evident at KK however the differences were less. Ear and stem leaf senescence pattern largely followed that of leaf senescence at both sites. There were significant positive effects of increasing seed rate on stem lodging ($5-45^\circ$ from the horizontal) and stem brackling at both sites and on stem leaning at CW only (Table 4.8).

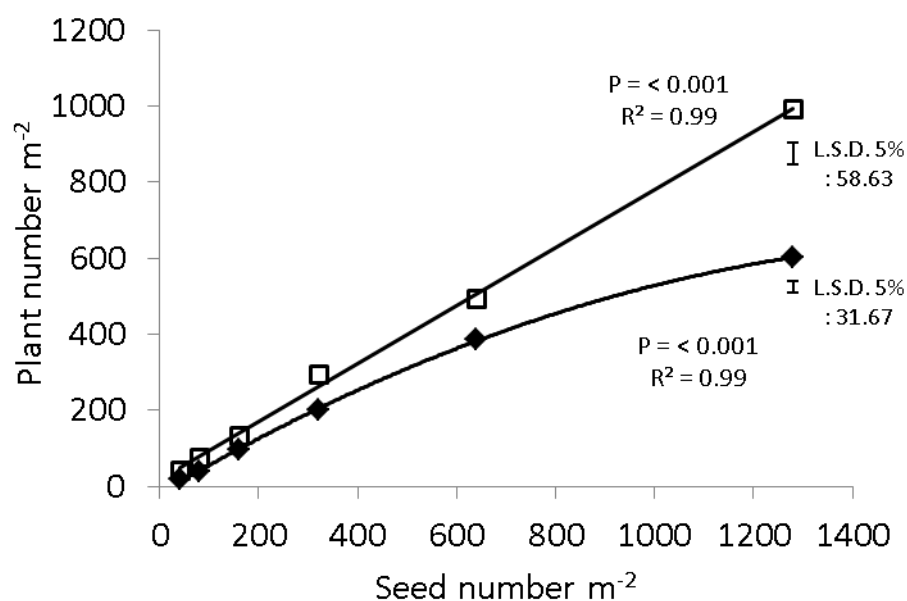


Figure 4.4. Linear and second order polynomial regressions fitted to plots of seed number m^{-2} versus plant number m^{-2} for CW (closed diamonds) and KK (open squares) in 2013. L.S.D. 5% (least significant difference at $P = 0.005$) for seed rate effect following ANOVA is also given for each site.

Table 4.7. Mean values of plant number m^{-2} at CW and KK for each seed rate treatment along with the standard deviation of the means. $n = 20$ (5 measurements \times 4 replicate plots per treatment)

	40 seeds m^{-2}	80 seeds m^{-2}	160 seeds m^{-2}	320 seeds m^{-2}	640 seeds m^{-2}	1280 seeds m^{-2}
CW 2013						
Mean plant no. m^{-2}	19	38	95	200	387	601
Standard deviation	15	21	27	75	76	104
KK 2013						
Mean plant no. m^{-2}	43	78	135	295	495	993
Standard deviation	20	28	56	60	96	139

Table 4.8. Mean values of % of shoots leaning, lodged, and brackled for seed rate treatments at CW and KK in 2013. Values are means of % scores for the whole plot area at harvest. *P* values and L.S.D. 5% (least significant difference at *P* = 0.05) values are for effects of seed rate following ANOVA. *ns* = non-significant. No shoots were lodged flat (45°-90°) at either site.

	40 seeds m ⁻²	80 seeds m ⁻²	160 seeds m ⁻²	320 seeds m ⁻²	640 seeds m ⁻²	1280 seeds m ⁻²	<i>P</i>	L.S.D. 5%	d.f.
CW 2013									
Shoots leaning (0-5°)	6.25	10	10	10	10	8.75	0.031	2.482	15
Shoots lodged (5-45°)	2.5	12	10	11.02	11.25	14	0.023	6.213	15
Shoots brackled	0	3.75	4.5	6.92	5.5	7.5	<0.001	2.495	15
KK 2013									
Shoots leaning (0-5°)	6.25	6.25	6.25	8.75	8.75	10	0.269	<i>ns</i>	15
Shoots lodged (5-45°)	4.5	7.5	8.8	17.5	20	21.2	0.021	11.18	15
Shoots brackled	4.2	4.8	6	9.5	15.8	13.8	0.047	8.5	15

4.3.3.2 Response of yield and yield components to seed rate

Yield, grain number and MGW values obtained from combine harvested grain samples are used here in preference to those obtained from and hand-threshed quadrat samples. Quadrats represented a limited area of plots and variation in crop establishment and growth within plots, particularly at lower seed rates, may have contributed to variation in mean values of yield components calculated using this method. Combine data were averaged across a 12 m x 2 m area and therefore provided a better estimation of yield and yield components to determine seed rate effects.

At both sites, there were significant positive effects of increasing seed rate on yield, grain number m⁻² and ear number m⁻² and significant negative effects on MGW,

grain number per ear and ear number per plant (P always < 0.001 ; Table 4.9). There was no significant effect of seed rate on harvest index at either site. At CW there was no significant effect of seed rate on harvest values of total biomass, ear biomass and stem plus leaf biomass, while at KK there was (P always < 0.001) where the 160 seeds m^{-2} treatment produced significantly higher biomass values for each portion than the 80 seeds m^{-2} did; there was no significant difference between 40 seeds m^{-2} and 80 seeds m^{-2} and no significant difference between seed rates greater than or equal to 160 seeds m^{-2} .

The effects of increasing seed rate on yield, grain number m^{-2} and MGW are illustrated in Figure 4.5 where linear plus exponential curves were fitted to each relationship. Separate curves were fitted for each site. Increases in both yield and grain number m^{-2} approached a plateau at approximately 200 seeds m^{-2} beyond which both variables increased with increasing seed rate but at a slower rate (L.S.D. 5% values for each site following ANOVA are given in Table 4.9). KK values of both yield and grain number were consistently higher than CW for any given seed rate. MGW decreased sharply with increasing seed rate, especially at CW, up to a seed rate of approximately 300 seeds m^{-2} at both sites. Thereafter MGW changed very little. MGW values were more conserved across seed rates at KK than CW.

Table 4.9. Mean values of yield, yield components and other harvest variables for six seed rate treatments at CW in 2013 and KK in 2013. *P* values and L.S.D. 5% (least significant difference at $P = 0.05$) values are for seed rate effects following ANOVA; ns = non-significant; DM = 100% dry matter; yield and MGW expressed at 85% DM;

CW 2013	40 seeds m ⁻²	80 seeds m ⁻²	160 seeds m ⁻²	320 seeds m ⁻²	640 seeds m ⁻²	1280 seeds m ⁻²	<i>P</i>	L.S.D. 5%	d.f.
Yield (t ha ⁻¹)	4.20	6.17	6.86	7.57	7.73	8.04	<0.001	0.99	15
Grain no. m ⁻²	7604	11834	13708	15786	16447	17208	<0.001	1538	15
MGW (mg)	53.92	51.15	48.58	46.82	45.87	45.61	0.002	3.76	15
Ear no. m ⁻²	544	587	764	741	1133	1361	<0.001	325	15
Grain no. ear ⁻¹	22.81	21.72	20.36	17.70	14.80	13.48	<0.001	3.11	15
Harvest Index	57.93	57.32	53.49	56.21	57.5	57.66	0.15	ns	15
Ear no. plant ⁻¹	30.2	15.5	8.1	3.5	3.0	2.3	<0.001	4.9	15
Total biomass (t ha ⁻¹ DM)	9.64	9.53	10.53	9.22	11.69	12.19	0.108	ns	15
Ear biomass (t ha ⁻¹ DM)	6.57	6.36	6.77	6.20	7.76	8.09	0.231	ns	15
Straw+leaf biomass (t ha ⁻¹ DM)	3.01	3.07	3.94	3.21	3.85	4.05	0.126	ns	15

See next page for KK 2013...

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KK 2013	40 seeds m⁻²	80 seeds m⁻²	160 seeds m⁻²	320 seeds m⁻²	640 seeds m⁻²	1280 seeds m⁻²	<i>P</i>	<i>L.S.D. 5%</i>	<i>d.f.</i>
Yield (t ha ⁻¹)	6.86	7.99	9.28	9.03	9.42	9.67	<0.001	0.58	15
Grain no. m ⁻²	13295	15975	18243	18706	19546	20125	<0.001	853	15
MGW (mg)	50.31	48.78	49.59	47.13	47.13	46.93	0.006	1.95	15
Ear no. m ⁻²	538	629	856	940	1260	1603	<0.001	183	15
Grain no. ear ⁻¹	23.56	21.36	19.10	18.74	14.66	11.31	<0.001	2.74	15
Harvest Index	56.30	49.00	55.20	56.50	55.20	55.10	0.347	ns	15
Ear no. plant ⁻¹	13.9	8.2	6.4	3.2	2.6	1.6	<0.001	4.6	15
Total biomass (t ha ⁻¹ DM)	10.01	10.01	12.79	13.18	13.39	13.73	<0.001	1.74	15
Ear biomass (t ha ⁻¹ DM)	6.55	6.73	8.11	8.79	8.56	8.80	<0.001	1.05	15
Straw+leaf biomass (t ha ⁻¹ DM)	3.49	3.77	4.60	4.90	5.09	5.33	<0.001	0.78	15

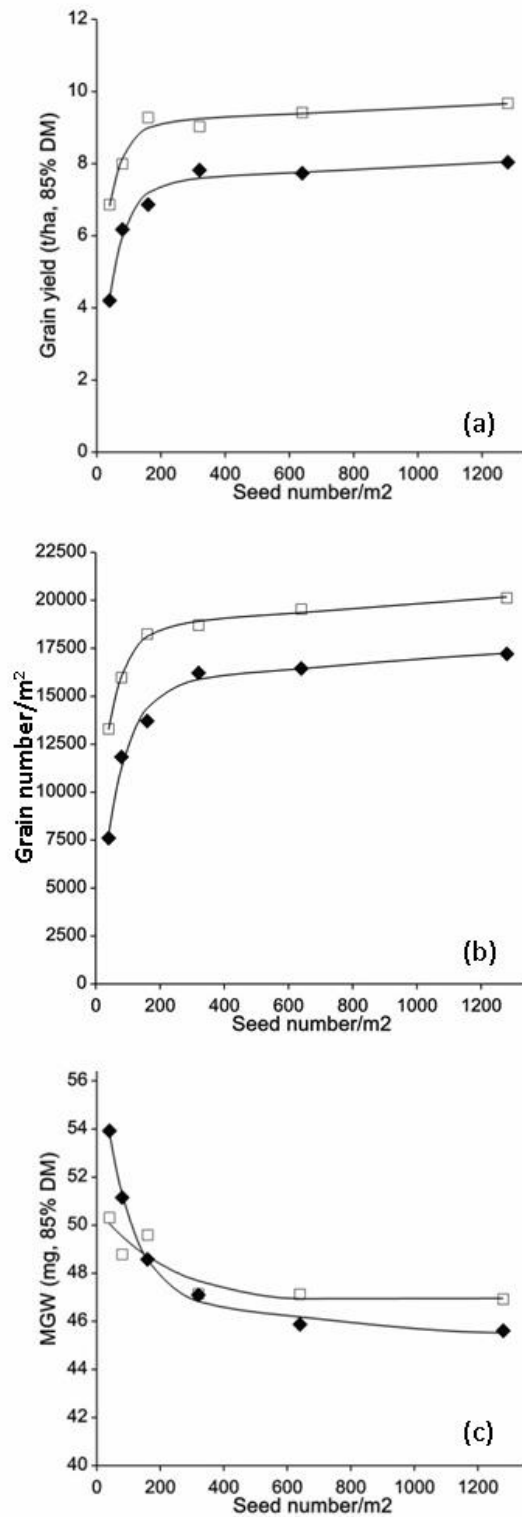


Figure 4.5. Linear plus exponential curves fitted to plots of seed rate versus (a) yield, (b) grain number m⁻² and (c) MGW. Data are replicate means of seed rate treatments at CW (closed diamonds) and KK (open squares) in 2013; DM = dry matter.

4.3.3.3 Trade-offs between yield components and the sub-components of grain number

Hand harvested quadrat data are used here in preference to combine yield data as they are the only source of data on the sub-components of grain number. The data are presented on a per plot basis given the variation in establishment that existed within plots and across treatments. Grain number m^{-2} was highly associated with yield ($P < 0.001$, $R^2 = 0.80$; Figure 4.6 (a)) across quadrats ranging in grain number from 7,725 to 21,500 grains m^{-2} . There was significant curvature in the relationship between grain number and MGW ($P = 0.027$) therefore a second order polynomial (quadratic) regression was fitted (Figure 4.6 (b)). Initially MGW declined as grain number was increased but then appeared to ‘bottom-out’ at approximately 48 mg as grain number continued to increase above $\sim 12,500 \text{ m}^{-2}$. However, grain number only explained a small proportion of the variation in MGW in this experiment ($R^2 = 0.18$). A split-line regression was fitted to the plot of ear number m^{-2} versus grain number m^{-2} where the slope of the second line was not significantly different to zero (Figure 4.6 (c)). The relationship appeared to plateau at a breakpoint of 1,018 ears and 18,322 grains m^{-2} . The strong negative relationship between ear number m^{-2} and grain number per ear ($P < 0.001$, $R^2 = 0.81$; Figure 4.6 (d)) explains this plateau in overall grain number above approximately 1000 ears m^{-2} .

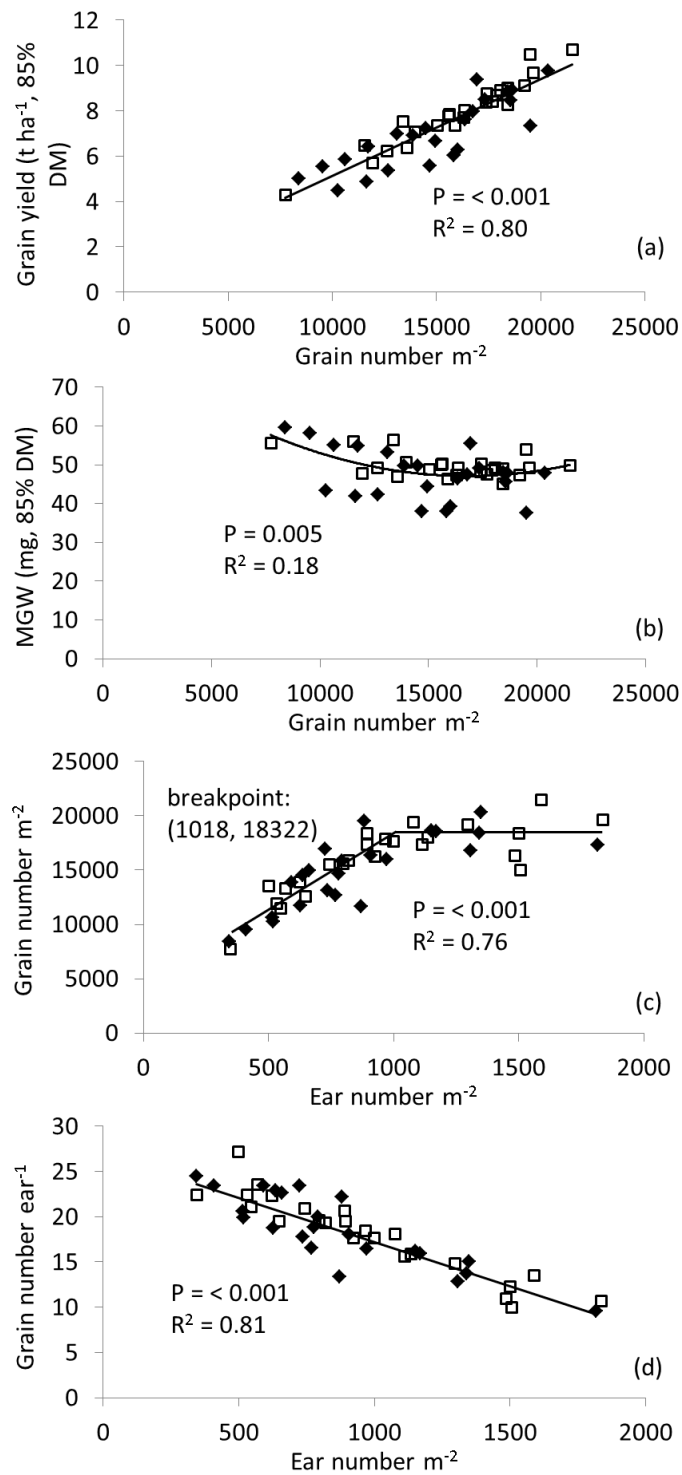


Figure 4.6. Plots of (a) grain number m⁻² versus grain yield; (b) grain number m⁻² versus mean grain weight (MGW); (c) ear number m⁻² versus grain number m⁻² and (d) ear number m⁻² versus grain number per ear. R^2 values following regression analysis. Data are individual plot values across six seed rates and four replicates at CW (closed diamonds) and KK (open squares) in 2013; DM = dry matter.

4.4 Discussion

4.4.1 Modification of photosynthetic assimilation capacity ‘early’ post-anthesis

Given that both shading and opening-up ‘early’ did not significantly reduce or increase MGW at harvest it is unlikely that MGW of barley is sensitive to variations in incident light during early grain development. The lack of effect was surprising as the literature suggests that GSC is determined by assimilatory capacity during the early post-anthesis period via effects on endosperm cell division (Bingham et al., 2007b; Brocklehurst, 1977; Cochrane and Duffus, 1983; Gleadow et al., 1982; Hasan et al., 2011; Nicolas et al., 1985).

Shading reduced net assimilate production by plants during the treatment period. Thus stem biomass was reduced relative to unshaded controls, as was the deposition of ethanol and water soluble carbohydrate reserves in the stem. By contrast, ear biomass and the concentration of low molecular weight sugars (mostly hexoses) in the grain were unaffected by shading. These results suggest that when assimilate production was reduced by shading during the first two weeks after anthesis, plants were able to maintain the concentration of important carbohydrate pools within the grain at the expense of storage reserve deposition. The maintenance of grain sugar concentrations does not appear to depend on the manner of their expression. When expressed on a tissue water basis, which has greater physiological relevance than expression per unit dry weight, sugar concentrations in the ear (water content of grains was not measured) were similarly unaffected by shading. Also, chaff sugar concentrations which were based on the same extraction matrix as grain sugars were also reduced while levels in the grain were maintained.

At the cellular level, metabolism responds to the concentration of metabolites in solution which may in turn drive the flux to the tissue (Jenner, 1970; Jenner, 1973, 1976; Jenner and Rathjen, 1972a). As such, the buffering of sugar concentrations in the grain may have maintained the process of endosperm cell division during the shading period and maintained the development of GSC resulting in a MGW similar to control levels at harvest. Fabian et al. (2011) and Jenner (1979) have shown in

wheat, that a reduction in MGW in response to a reduction in assimilate supply for a short period early post-anthesis was likely to be a consequence of a reduced endosperm cell number (Jenner, 1979) and not simply a consequence of depleted levels of assimilate per grain for subsequent grain filling. It is therefore plausible that maintenance of sugar concentration in the grain during the shading period in the present study maintained GSC and hence MGW at harvest. Fabian et al. (2011) and Jenner (1979) highlighted the importance of the early post-anthesis period for establishing GSC but these experiments were carried out in controlled environments. The divergence of the present study from these findings may mean that the same limitations are not present in the field, at least in the environmental conditions where this study was conducted.

The lack of effect of row-opening on MGW might be because endosperm cell division was insensitive to the additional light availability per plant and the increase in photo-assimilate supply that would be expected to occur. Grain sugar concentrations may have been maintained similar to the shading experiments with additional assimilate deposited as storage reserves. However, it is also plausible that had the treatment increased GSC and the crop may have become source-limited and unable to fill its grains fully, especially during the dull grain filling period in 2012. By contrast transition between source and sink limitation cannot explain the fact that MGW was not reduced by shading ‘early’ in 2012 and again in 2013.

Results of the early shading experiment in 2013 also provided some evidence of a possible up-regulation of photosynthesis after shading was removed. Shading reduced biomass accumulation by 2.6 t ha^{-1} during the treatment period, but the difference in total biomass at harvest was less than 1.0 t ha^{-1} . Differences between shaded and unshaded plants in stem soluble carbohydrate concentrations also decreased after shades were removed suggesting some compensatory adjustment in photosynthetic activity of previously shaded plants.

Varying seed rate in the shading experiment in 2013 did not result in higher grain numbers than were achieved in 2012 as plots suffered from poor establishment (low plant numbers m^{-2}) due to the unusually cold and damp environmental conditions that occurred immediately post-sowing (discussed in more detail in Chapter 2).

Nonetheless, increasing seed rate had a significant effect on MGW and all other harvest variables measured. It can therefore be assumed that seed rate modified the source:sink balance throughout the season. If assimilate supply after anthesis was important in regulating grain development, crops with a lower source:sink ratio would be expected to be more sensitive to reductions in light interception imposed by shading. While there was a significant (negative) seed rate effect on MGW at harvest, there was no seed rate x shading interaction effect indicating that plants with differing final MGW were equally insensitive to variations in net assimilation during early grain development. Assuming that post-anthesis assimilate supply was sufficient for grain filling, this implies that differences in MGW were determined by the storage capacity set prior to anthesis, perhaps through a reduced carpel weight.

4.4.2 Further seed rate treatments

The consistently higher yield and grain number achieved across seed rates at KK than at CW in 2013 was likely a consequence of the superior plant establishment at that site. However the plateau in yield and grain number at higher seed rates was not a consequence of a plateau in plant number m^{-2} – plant number increased linearly at KK and curvilinearly at CW in response to increasing seed rate. Across all plots at both sites, grain number m^{-2} plateaued at around 18,000 m^{-2} above an ear number of approximately 1000 m^{-2} when reductions in grain number per ear became so great as to counteract the influence of additional ear numbers. The similar response of yield and grain number m^{-2} to increasing seed rate is unsurprising given that grain number largely determined yield across the two sites.

Evidence from Chapter 3 suggests that grain number is set at anthesis. If GSC is also determined prior to anthesis then it is possible that both of these sink components are determined at the same time. The tight dependence of both grain number and GSC on the available resources during a specific developmental period has important implications for productivity particularly if a trade-off between the two exists (Gambín and Borrás, 2010). Total resources available around the seed set period may be proportionally allocated to produce either many small seeds or few larger individual seeds depending on the crop species (Gambín and Borrás, 2010). Barley is

a species that produces relatively small numbers of large grains compared to many other species. Grain number per unit area was restricted at higher ear numbers by a decreasing grain number per ear at the CW and KK seed rate experiments in 2013. It is possible that this was a consequence of the prioritization of the reproductive fitness (size) of harvested grains as hypothesized by (Sadras, 2007) whereby grain number was adjusted to ensure that all grains could fill to a pre-established genetically determined lower limit of storage capacity (Gambín and Borrás, 2010). This might involve resource-based mechanisms in which floret survival was regulated by assimilate availability and/or crop growth rate during late stem extension (Sadras and Slafer, 2012).

Assimilate anticipation for grain filling is implied (Gambín and Borrás, 2010) and it appears that a trade-off between grain number and GSC occurred via a reduction in grain number per ear at high ear numbers. There is a large degree of overlap between the determination periods of grain number per ear and ear number causing an additional trade-off between these sub-components (Baethgen et al., 1995; Wade and Froment, 2003; Willey and Holliday, 1971). This happens during stem extension when there is a large increase in total crop growth rate and demand for assimilate (Kirby, 1977). González et al. (2011) have shown in wheat that floret death and survival were linked to the onset of rapid ear growth. Spikelets in barley and wheat are particularly sensitive to assimilate availability during this phase of development (Grashoff and d'Antuono, 1997; Willey and Holliday, 1971) and as such some will die at a young age due to a shortage of photosynthate (Arisnabarreta and Miralles, 2008a; Kirby, 1977; Kirby and Appleyard, 1984; Waddington and Cartwright, 1983). At the higher ear number plots at CW and KK a potential restricted canopy size or a plateau in the amount of light intercepted by the crop (less light intercepted per grain) may have resulted in insufficient photosynthesis to support additional spikelets. Nitrogen availability also has the potential to influence spikelet survival in barley (Baethgen et al., 1995). Without sufficient data it is not possible to identify what became limiting. Aside from assimilate availability, a pre-emptive signaling strategy may be responsible for the death of florets. Florets in wheat can be particularly sensitive to environmental conditions, namely PAR interception (Fischer, 1985; Reynolds et al., 2009) and photoperiod (Gambín and Borrás, 2010)

and particularly during the relatively narrow developmental window of rapid spike growth (Abbate et al., 1997; Fischer, 1985). Additionally, barley and wheat florets are sensitive to stress during meiosis (nuclear and cell division in preparation for anthesis) and this can result in sterility or decreased grain set (Kirby and Appleyard, 1984). The reduction in grain number per unit area in response to the early post-anthesis shading treatment in 2013 may be a consequence of such a decreased grain set and highlights that grain number is potentially more sensitive to events around anthesis than MGW.

4.5 Conclusion

The results highlight that under field conditions in Ireland, MGW was insensitive to large variations in light availability and hence net assimilation during early grain development. The crop responded to variations in net assimilation by altering deposition of stem storage reserves whilst maintaining soluble sugar concentrations in the grain. As a consequence, it is likely that GSC, namely endosperm cell division, was maintained during the shading and opening up treatments.

Results suggest that early grain development is not an important period for determining GSC and that the pre-anthesis period may be more important thus presenting a potential trade-off between grain number and GSC. There were trade-offs between yield components when plant numbers were varied. MGW decreased up to a grain number of approximately 12,500 m⁻² beyond which it remained relatively conserved. There was also a trade-off between the sub-components of grain number. A strong negative relationship between ear number and grain number per ear resulted in a plateau in overall grain number of approximately 18,000 grains m⁻²; this was achieved with an ear number of approximately 1000 ears m⁻². This supports the view that grain numbers may be adjusted according to resource availability in a way that helps conserve MGW (Gambín and Borrás, 2010; Sadras, 2007). Breaking the strong negative relationship between ear number and grain number per ear may require additional assimilate availability during stem extension.

Chapter 5 **General Discussion**

5.1 Estimates of yield potential from season long values of resource capture and utilisation

Crops assimilate carbon and accumulate dry matter at a rate that is proportional to the amount of radiation intercepted (Biscoe and Gallagher, 1977; Monteith, 1972, 1977). Yield potential can, therefore, be expressed as a function of the amount of incident photosynthetically active radiation (incident PAR), the percentage intercepted by the canopy (% PAR intercepted), the efficiency with which that energy is converted into dry matter (RUE) and the fraction of dry matter partitioned into harvested components (harvest index (HI)) (Newton et al., 2011; Reynolds et al., 2005). Maximum yield potential for Irish conditions can be estimated in this way by using maximum values of % PAR intercepted, RUE and HI achieved across the 9 site/seasons of crops in Chapter 2 and average values of incident PAR. This approach assumes that these determinants of yield are independent of each other and that it might be possible to combine the best of each under appropriate crop management. The highest % PAR intercepted value from emergence to senescence of 76 % was achieved at CK 2011. The maximum RUE was 3.05 g MJ⁻¹ at WX 2012 and the maximum HI was 0.62 at CW 2013. When these data are combined with the short-term average incident PAR at the three sites (solar radiation data available from 2008 to 2013 only) between the dates of emergence and senescence at CK 2011, yield potential was estimated at 15.37 t/ha at 85 % dry matter.

It is likely that this estimate could be improved upon by increasing RUE and the % interception of seasonally available PAR, but not HI. The consensus in the literature is that current genetic material is approaching the upper limit of HI of approximately 0.50-0.60 (Cassman, 1999; Hay, 1995; Jenkins, 1985; Miralles and Slafer, 2007; Naylor et al., 1998; Reynolds et al., 2005; Riggs et al., 1981; Slafer et al., 2005), therefore further HI increases above the value of 0.62 are unlikely. Theoretical limits to RUE have been estimated to be between 3.0 and 5.2 g biomass MJ⁻¹ absorbed PAR depending on the quantum requirement for photosynthesis (10-30 photons mol⁻¹ CO₂) which varies with environment (Reynolds et al., 2000). It should be noted that theoretical maximum values based on intercepted, rather than absorbed, PAR would

be a little lower. Given that barley crops in Ireland have RUEs at the bottom end of this range there would appear to be some potential for increasing RUE in the field through genetic improvement of varieties (Reynolds et al., 2000). Increasing the amount of seasonally available PAR intercepted could be achieved by increasing early season interception via earlier canopy development or late season interception via prolonged canopy lifespan.

Earlier sowing as a method of increasing the amount of PAR intercepted during the crop lifecycle must be approached with caution due to the higher risk of poor crop establishment if cool and wet conditions prevail as witnessed at CW 2013 where low plant numbers translated into low shoot numbers, low grain numbers, and ultimately a yield that was 36 % lower than the 9 site/season average (Chapter 2). Season long PAR interception could also potentially be increased by prolonging the crop lifespan i.e. delaying senescence. While CK 2011 intercepted the highest proportion of incident PAR from emergence to senescence, it also had the second earliest date of senescence, which was 5 days earlier than the median date for the 9 site/seasons. If the lifespan of the CK 2011 canopy could have been prolonged for a further 5 days, thus increasing the crop lifecycle and available incident PAR, then the yield potential estimate of 15.37 t/ha increases to 16.00 t/ha (provided the same whole-season % PAR interception is maintained). Prolonging canopy life span to increase incident PAR might be achieved with the use of fungicides with ‘stay-green’ physiological effects or late season N, but would need to be achieved without delaying harvesting operations given that harvesting grain with a low enough moisture content for storage is already challenging in Irish autumn weather conditions.

5.2 Considering the sink limitation of yield formation

The type of analysis, outlined above, based on season long averages of % PAR interception, RUE and HI is useful in quantifying the limits imposed on yield potential by the radiation environment and potential resource capture, but it is simplistic. It fails to take into account the relationship between source and sink and the role of plant development in imposing sink limitations on yield. For example,

although increasing post-anthesis canopy duration might theoretically increase PAR interception and thus assimilate supply for grain filling, it will not result in an increase in yield if there is insufficient sink capacity for starch storage.

Grain number is highly associated with barley yield across environments (Abeledo et al., 2003; Baethgen et al., 1995; Bingham et al., 2007a; Blake et al., 2006; del Moral et al., 2003; Gallagher et al., 1975; Peltonen-Sainio et al., 2007; Serrago et al., 2013). High grain numbers were achieved across nine site/seasons in Ireland (Chapter 2). It might be expected that in such an environment crops could become source limited resulting in a weak or non-existent relationship between grain number and yield. However this was not the case and a strong relationship between grain number and yield was demonstrated across several sites, seasons and seed rate treatments throughout this thesis. This indicates that grain number largely determined yield. Grain weight remained relatively conserved when compared to grain number (see Chapter 3, section 3.1 and references therein). Further, manipulating source:sink ratios in barley and other small grain crops during grain filling have been found to have less effect on grain weight than the change in ratio imposed (Beed et al., 2007; Borrás et al., 2004; Estrada-Campuzano et al., 2008; Grashoff and dAntuono, 1997; Habgood and Uddin, 1983; Jenner, 1979; Serrago et al., 2013; Willey and Holliday, 1971) and this has also been demonstrated in Chapter 3. Evidence suggests that a sink limitation of barley yield, or a co-limitation by source and sink, is the norm rather than source limitation. This supports evidence provided in the literature of a surplus of assimilate for grain filling from post-anthesis photosynthesis (Dreccer et al., 1997; Richards, 2000; Serrago et al., 2013; Slafer and Savin, 1994) and pre-anthesis storage reserves (Beed et al., 2007; Fabian et al., 2011; Foulkes et al., 2007; Serrago et al., 2013; Yoshida, 1972). However, an important question is the extent to which sink limitation operates during grain filling (Bingham et al., 2007a), because increasing sink capacity might place the crop at greater risk of source limitation unless corresponding increases in post-anthesis assimilate supply can be made. There is evidence that in Ireland barley MGW can be reduced significantly when major reductions in incident light are imposed by shading during rapid grain filling (Chapter 3). While % reductions in MGW were less than the % reductions in incident PAR, this implies that the sink limitation expected under ambient conditions has

been replaced by source limitation during shading. Thus, if grain numbers are to be increased, it is important to establish that there will be sufficient assimilate available post-anthesis for them to fill adequately. Increases in assimilate supply could occur via increases in post-anthesis PAR interception, RUE or utilisation of soluble carbohydrate reserves - the relative contribution of each to grain filling varied across the 9 site/seasons in Chapter 2.

Excluding CW 2013, where canopy closure did not occur due to the poor and delayed crop establishment, the average GAI at anthesis of 5.7 across the other 8 site/seasons of crops in Chapter 2 resulted in greater than 93 % PAR interception. Thus, there is little scope for significant improvement in % PAR interception during early grain development and grain filling. By contrast overall increases in post-anthesis PAR interception could be achieved by delaying canopy senescence as described above where methods to increase season long PAR interception were discussed.

There is evidence that post-anthesis RUE might be regulated in response to changes in sink demand for assimilates. For the shading x seed rate experiment discussed in Chapter 4, the mean increase in total above ground dry matter per shoot from 14 days after anthesis until physiological maturity (GS 87) was 37 % greater for crops that had been shaded for the previous 14 days than for unshaded controls. The fact that such an increase in dry matter production was possible in the absence of any increase in canopy duration suggests that in normal ambient conditions post-anthesis RUE was down regulated, and given a higher sink capacity, a higher RUE could be maintained. Evidence from Chapter 2 and Bingham et al. (2007a) has also shown that RUE may be down-regulated at certain site/seasons during grain filling perhaps as a result of feedback inhibition of photosynthesis by a limited sink demand for assimilates. In the seed rate x shading experiment there was no difference in MGW between shaded and unshaded treatments at harvest. It is therefore plausible that each shoot could have sustained 37 % more grains per ear than were achieved. This translates into an additional 5.7 grains per ear over and above the mean control value of 15.7. At the control ear number of 909 m⁻², these crops could have potentially sustained an additional 5,215 grains m⁻². However CW 2013 was a particularly low

yielding site/season due the already mentioned poor crop establishment that occurred and a control value of just 12,475 m⁻² was achieved in the shading x seed rate experiment. Sink limitation may have been greater at this site/season and thus the potential up-regulation of RUE may have been greater than what would be expected at more normal site/seasons. The above estimates were based on mean data from three seed rates. The low, standard and high seed rates in this experiment achieved 9,380, 12,283 and 14,287 grains m⁻² respectively and their corresponding potentials for up-regulation of RUE calculated as above were 50 %, 39 % and 12 %. This suggests that as sink capacity increased, the potential for increasing RUE decreased. If the relationship is assumed to be linear then under these experimental conditions the capacity for up-regulation would be zero at a grain number of 15,929 m⁻². However, this apparent threshold is also likely to be influenced by the light environment and variations in canopy architecture. There was evidence of a decline in RUE towards the end of grain filling at WX in 2011 (and arguably 2012) and CK 2011, possibly resulting from surplus photosynthetic capacity, even though these crops had grain numbers between 17,276 and 23,317 m⁻².

The values of potential up-regulation of RUE for the standard and high seed rates in Chapter 4 are in line with increases in RUE reported for spring wheat whose grain number per ear was increased experimentally by a light treatment during booting (Reynolds et al., 2005). An average increase of 3 grains per ear was associated with an increase in yield of 25 %, of crop biomass at harvest of 22 %, and in rate of photosynthesis of the flag leaf during grain filling of 10 % (Reynolds et al., 2005). Thus, relief of feedback inhibition of photosynthesis through an increased sink capacity may help sustain grain filling if grain numbers and potential grain size of barley were to be increased. The contribution of storage reserves to grain filling can vary with environment, season and sink size (Bingham et al., 2007a; Gallagher et al., 1975). The amount of water soluble carbohydrate reserves present in the stems of wheat and barley crops around anthesis can vary from 2-3 t ha⁻¹ of dry matter (2.4-3.5 t ha⁻¹ at 85% dry matter) (Bingham et al., 2007a; Foulkes et al., 2002; Foulkes et al., 2007; Gaunt and Wright, 1992; Grashoff and d'Antuono, 1997; Shearman et al., 2005).

The average interception of incident PAR from anthesis to senescence across the 9 site/seasons of crops in Chapter 2 was 83%. The average incident PAR for the three sites (2008-2013; CW, WX, CK) from the median date of anthesis to the median date of senescence was 297 MJ m⁻². Combining these data with the mean RUE of 2.68 g MJ⁻¹, potential post-anthesis dry matter production was estimated as 7.71 t/ha at 85% dry matter. The amount of soluble sugars present in stems at anthesis was estimated at 1.14 t/ha at 85% dry matter (DM) averaged from crops of standard seed rate at CW and KK in 2013. If this is assumed that all of these storage reserves can be remobilized then a total of 8.85 t/ha at 85% DM is available for grain filling. If this is divided by the mean MGW from the 9 site/seasons (reduced by 3.5 mg/grain to account for the husk weight already present at anthesis) then a potential grain number of 20,635 m⁻² would be required to utilise all of the potential post-anthesis assimilate available. Including the husk weight with the post-anthesis growth relates to a potential yield of 9.58 t/ha at 85% DM.

Assuming an increase in post-anthesis canopy duration of a modest 3 days (so as not to unduly delay harvesting operations), an increase in RUE of 10% by relief of feedback inhibition of photosynthesis, and an increase in availability of stem soluble carbohydrate reserves to 2.4 t ha⁻¹ at 85% DM, potential assimilate availability for grain filling increases from 8.85 t ha⁻¹ to 11.36 t ha⁻¹ at 85% DM. This assumes that the same % of the increased PAR available is intercepted by the crop. If the same MGW is also assumed then the estimated potential grain number required to utilise this assimilate rises from 20,635 m⁻² to 26,481 m⁻² and the estimated yield potential rises from 9.58 t ha⁻¹ to 12.29 t ha⁻¹ at 85% DM when the husk weight present at anthesis is accounted for. These values of grain number and yield are both 44% higher than the 9 site/season mean values from Chapter 2 of 8.52 t/ha and 18,419 grains m⁻² which are representative of current management practices in Ireland.

5.3 Routes to increasing grain number m⁻²

As % PAR interception by well managed canopies shortly after anthesis is generally greater than 93 % (Chapter 2), increasing grain numbers to increase sink capacity

would be associated with an unavoidable decrease in the amount of PAR intercepted per unit grain number during the early grain development period. However, experiments in Chapter 4 showed that MGW was insensitive to variations in PAR interception during this period of endosperm development, because soluble sugar concentrations in the grain were maintained at the expense of storage reserve deposition in the stems. Had this not been the case, then possible reductions in grain storage capacity (potential grain size) resulting from the lower PAR interception per grain could have negated the effects of increasing grain number on overall sink capacity.

Shoot survival is an important determinant of grain number and achieving high shoot numbers of adequate size and weight at GS31 may be an appropriate target for establishing a high yield potential crop (Chapter 2). However once high potential ear numbers are secured ($> 1000 \text{ m}^{-2}$), breaking the negative relationship between ear number and grain number per ear may hold the key to further increasing grain number and hence yield potential (Chapter 4). The fact that there was no abortion of grains following post-anthesis shading confirms that grain number is largely determined pre-anthesis (Chapter 3). A small reduction in grain number m^{-2} (8%) was found in response to early shading in 2013 (Chapter 4) however this was likely a reduction in grain set in tillers or spikelets that reached anthesis after the treatment was imposed rather than a post-anthesis abortion or down-regulation of grain number. Given the insensitivity of MGW to variations in assimilate availability early post-anthesis it is likely that GSC is also determined pre-anthesis (Chapter 4). Furthermore, since pre-anthesis stem soluble carbohydrate reserves are an important source of post-anthesis assimilate then a potential three-way trade-off exists between deposition of reserves and the establishment of grain numbers and GSC, which most likely manifests itself during stem-extension. The stem extension phase of development is one where there is a high demand for assimilates and the co-determination of grain number and GSC during this period could result in a trade-off between these two sink components during stem extension whereby grain number is adjusted in response to an assimilate shortage to ensure that a fewer number of florets with a larger storage capacity survive (Gambín and Borrás, 2010) thus prioritising reproductive fitness (Sadras, 2007).

It has been argued that focusing on traits associated with promoting ear growth during stem extension may be valuable when breeding for further increases in yield potential in wheat and barley through its influence on floret survival (González et al., 2011). A similar strategy may be employed to increase grain number in barley crops where high ear numbers are already achieved. Grain number per ear is more conserved in barley than in wheat because each spikelet contains only one floret as opposed to several in wheat. However there is still scope for increasing grain number per ear in barley given that during the floret initiation phase of apical development a maximum of 35-45 spikelet primordia per shoot are formed (Gallagher et al., 1976; Kirby, 1977; Kirby and Appleyard, 1984) and resulting grain number per ear at harvest can range from just 11-24 per ear depending on shoot number (Chapter 4). Reducing spikelet mortality in high ear number crops may thus provide a realistic means of achieving the 44% increase in grain number required to realise the yield potential as estimated above.

Incident radiation and assimilate supply during stem extension can influence spikelet survival (Arisnabarreta and Miralles, 2008a; Borras et al., 2009). Increasing spikelet growth during the stem-extension period by lengthening its duration is one possible approach to enhancing spikelet survival (Abeledo et al., 2003; Miralles et al., 2001; Miralles et al., 2000; Miralles and Slafer, 2007; Reynolds et al., 2009). Slower floret development results in more potential grain sites (Miralles et al., 1998; Miralles and Slafer, 2007). Extending the period of stem elongation by exposure to short photoperiod can result in an increased number of fertile florets in wheat (Slafer et al., 2001) and can be achieved by selecting for a higher sensitivity to photoperiod (Slafer et al., 2005). The genes responsible for sensitivity/insensitivity to photoperiod are well known and easily manipulated (Borras et al., 2009; Slafer et al., 2005). However, lengthening the stem elongation period would need to be achieved without constricting the grain filling period and/or resulting in a late harvest if the higher grain numbers are to achieve a marketable grain weight (Reynolds et al., 2009). Plant growth regulators applied around stem extension have had limited success in promoting spikelet survival (Ma and Smith, 1991, 1992; Rajala and Peltonen-Sainio, 2001, 2002; Waddington and Cartwright, 1986; White, 1989).

As growth rate of spikelets rather than developmental cues may be key to their survival (González et al., 2011), increasing pre-anthesis RUE thereby making more assimilate available to increase ear mass during stem extension may avoid unnecessary spikelet mortality during this period (Reynolds et al., 2009). This may be achieved by breeding for varieties with a more erect leaf growth habit to prevent light saturation of the upper leaves of the canopy thus increasing RUE. Alternatively, selecting for shorter stems, shorter leaf sheaths or shorter awns may allow the assimilate otherwise utilised in the formation of these structures to become available to support spikelet growth during stem extension and reduce spikelet mortality. However, the impact of this on reducing post-anthesis photosynthetic capacity would need to be considered. Increased nitrogen fertiliser application at stem-extension is one potential crop management approach to reduce spikelet mortality during stem extension. Traits aimed at increasing nitrogen utilisation efficiency (NUE) during stem extension may also help to avoid unnecessary spikelet mortality. Achieving increases in NUE may be more appropriate than increasing rates of nitrogen application given the EU legislation restricting the amount of nitrogen fertiliser that can be applied in any one season.

The strategies aimed at increasing yield potential discussed here are based on experimental work carried out using the two-row type barley variety Quench. Quench features in the parentage of many of the varieties currently on the Irish Department of Agriculture, Food and the Marine Recommended Variety List for spring barley and as such the findings of this thesis are most likely applicable to the Irish spring barley crop as a whole. Alternative strategies may be required for six-row type barleys (six-row types are generally winter sown) given their reduced tillering capacity and greater number of grains per ear compared to two-row types. It is unknown whether six-row type barleys are in closer source:sink balance than two-row types. If this were the case strategies aimed at increasing the yield of six-row types may need to be targeted at increasing assimilate available for grain filling. Attempts by breeders to combine the best characteristics of both types have been largely unsuccessful with crosses resulting in low numbers of grains per ear and unacceptably small grains (Riggs and Kirby, 1978).

5.4 Other considerations and conclusions

Improved lodging resistance will be required to counter the increased lodging risk arising from continued yield increases (Berry et al., 2007). High tillering genotypes tend to have a smaller stem diameter and a tendency to lodge (Simmons et al., 1982). In crops of very high shoot number, competition for light may lead to taller and thinner stems with a greater risk of lodging (Pinthus, 1973). Any modification of assimilate partitioning to achieve heavier more fertile ears must ensure a sufficient allocation of dry matter to stem structure and root growth to ensure sufficient stem strength and anchorage (Foulkes et al., 2011; Reynolds et al., 2009) as these traits may be more important in reducing lodging risk than selecting for a reduced crop height (Berry et al., 2004). The implications for grain nitrogen and hence grain protein concentration of further increasing yield potential are unclear, however, strategies to improve yield that maintain grain weight may also maintain grain nitrogen concentration (Blake et al., 2006). A delayed mid-late season application of nitrogen fertiliser may be required to maintain grain nitrogen concentration at higher grain numbers but an increase in nitrogen use efficiency and nitrogen remobilisation within the plant may be a more important consideration given the socio-economic restrictions on nitrogen application rates. It is unlikely that an increase in screenings (grains < 2,25 mm dia.) would occur if increases in grain number do not exceed the amount required to match the estimate of potential assimilate availability for grain filling in section 5.2. Also, experiments in Chapter 4 showed that a reduction in grain storage capacity at these higher grain numbers is unlikely. Water and nutrient availability may become constraints to yield potential as higher grain numbers and yields are achieved and if so improvements in the efficiency of use of these resources by crops will be required. Donald (1968) speaks of a crop ideotype that makes a minimum demand on resources per unit of dry matter produced and has sufficient sink capacity to accept all available assimilate. The use of optimal germplasm must be considered as a powerful and necessary tool for maximising yield potential in a given environment and the design of ideotypes requires a knowledge of what

attributes must be modified at particular developmental stages (Serrago et al., 2013). This thesis has highlighted the high yield potential of the Irish climate and identified what is required to achieve high yields consistently using a high yield potential cultivar (cv. Quench). To increase yield potential an increase in grain sink capacity is required. Grain number per unit area and grain storage capacity were shown to be insensitive to variations in assimilate availability post-anthesis. It is therefore likely that both of these sink components are determined pre-anthesis. Given the high demand for assimilate during stem-extension and the relatively conserved nature of grain weight it is likely that a trade-off between both sink components occurs in an attempt to prioritise the reproductive fitness of the harvested grains (Sadras, 2007). Once high ear numbers are achieved increasing assimilate production and partitioning to ears during stem extension, either through increases in the duration of stem extension or RUE, may enable larger grain numbers to be produced whilst maintaining or increasing individual grain storage capacity and deposition of stem storage reserves.

Future research should, therefore, focus on ways to increase RUE during stem extension. This might require further improvements in canopy architecture or changes to photosynthetic efficiency such as the carboxylation efficiency of rubisco and reduced light saturation of upper leaves. This approach to increasing grain numbers would be facilitated by an improved understanding of the molecular processes regulating spikelet mortality and the mechanistic link between mortality and carbohydrate supply to the ear. Currently our understanding of what controls potential grain size is also poor and thus a better knowledge of the regulation of potential size at the tissue and molecular level might enable possible trade-offs between grain number and grain storage capacity to be uncoupled.

Improvements in yield must be sought without correspondingly large increases in fertilizer N requirement. Thus research is required to understand the factors that govern N utilisation efficiency during stem extension and grain number formation. Optimisation of N remobilisation after anthesis is then required to maximise the duration of photosynthetic activity whilst providing an adequate supply of N for the

grain so that high yields can be coupled to the grain N concentrations needed by end users.

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Appendix 1 Presentations, articles and posters

Kennedy, S. P., Spink, J. H., Bingham, I. J. 6th April 2011. Identifying constraints to increasing yield potential in barley. Oral presentation at the SAC Postgraduate Research Conference, Edinburgh, UK.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 14th April 2011. Identifying constraints to increasing yield potential in barley. Oral presentation as part of the Student Seminar Series, Teagasc, Oakpark, Carlow, Ireland

Kennedy, S. P., Bingham, I. J., Spink, J. H. 1st February 2012. Explaining spring barley yields in 2011. Oral presentation to a group of growers, agronomists and Teagasc staff as part of a project meeting at Teagasc, Oakpark, Carlow, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 23rd February 2012. Explaining spring barley yields in 2011. Oral presentation to a group of undergraduate agricultural students at Teagasc, Oakpark, Carlow, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 29th February 2012. Explaining spring barley yields in 2011. Oral presentation at the Agrometerology Conference 2012, Customs House, Dublin, Ireland.

Kennedy, S. P., Spink, J. H., Bingham, I. J. 21st March 2012. Limitations to yield potential in barley. Poster presentation at the SAC Postgraduate Research Conference, Edinburgh, UK.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 3rd December 2012. Identifying constraints to increasing yield potential in barley. Oral presentation as part of the Student Seminar Series, Teagasc, Oakpark, Carlow, Ireland

Kennedy, S. P., Spink, J. H., Bingham, I. J. 20th March 2013. Identifying constraints to increasing yield potential in barley. Oral presentation at the SAC Postgraduate Research Conference, Edinburgh, UK.

Kennedy, S. P., Spink, J. H., Bingham, I. J. 25th March 2013. Barley grain number response to a post-anthesis reduction in assimilation capacity. Oral presentation at the AAB Crop Resource Efficiency and Field Phenotyping Conference at Grantham, Lincolnshire, UK.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 3rd April 2013. Identifying constraints to increasing yield potential in barley. Oral presentation as part of the Teagasc agronomists training day at Teagasc, Oakpark, Carlow, Ireland.

Kennedy, S. P., Spink, J. H., Bingham, I. J. 25th April 2013. Physiology of yield in spring barley. Oral presentation to a group of undergraduate agricultural students at Teagasc, Oakpark, Carlow, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 26th June 2013. Spring barley growth, development and yield. Oral and poster presentations to various groups of growers, agronomists and others at the Teagasc Oakpark Crops Research Centre Open Day 2013, Carlow, Ireland.

Kennedy, S. P., Spink, J. H., Bingham, I. J. 28th October 2013. Grain number m⁻² in barley; how much is too much? Poster presentation (best poster winner) at the Teagasc Walsh Fellowship Seminar at the RDS, Dublin, Ireland.

Kennedy, S. P., Spink, J. H., Bingham, I. J. 2014. Grain number in barley. T-Research, Volume 9, no. 1, p 10. Teagasc, Oakpark, Carlow.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 9th January 2014. Variation in crop growth and yield potential of spring barley. Oral presentation as part of the Teagasc agronomists training day at Teagasc, Oakpark, Carlow, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 14th January 2014. Variation in crop growth and yield potential of spring barley. Teagasc Cork Tillage Conference 2014, Cork, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 20th January 2014. Variation in crop growth and yield potential of spring barley. Teagasc Wexford Tillage Conference 2014, Enniscorthy, Wexford, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 23rd January 2014. Variation in crop growth and yield potential of spring barley. Teagasc Carlow Tillage Conference 2014, Tullow, Carlow, Ireland.

Kennedy, S. P., Bingham, I. J., Spink, J. H. 27th January 2014. Identifying constraints to increasing yield potential in barley. Oral presentation as part of the Student Seminar Series, Teagasc, Oakpark, Carlow, Ireland

Kennedy, S. P., Bingham, I. J., Spink, J. H. 30th January 2014. Variation in crop growth and yield potential of spring barley. Oral presentation at the National Tillage Conference 2014, Kilkenny, Ireland.

O'Donovan, T. 11th February 2014. Grain numbers hold the key to improving spring barley yield. Article in the Irish Independent Farming Section, Independent News and Media, Dublin.

Spink, J. H., Kennedy, S. P. 25th January 2012. Explaining cereal yields in 2011. Oral presentation delivered by John Spink at the National Tillage Conference 2012, Kilkenny, Ireland.

Spink, J. H., Kennedy, S. P. 31st January 2013. Cereal yields in 2012 – what went wrong? Oral presentation delivered by John Spink at the National Tillage Conference 2013, Kilkenny, Ireland.

Spink, J. H., Kennedy, S. P., Kildea, S. 2013. Cereal Yields in 2012 – what went wrong? T-Research, Volume 8, no. 1, pp 34-35. Teagasc, Oakpark, Carlow.

Spink, J. H., Kennedy, S. P., Forristal, P. D. 5th December 2013. Minimising the impacts of extreme weather events on tillage production. Oral presentation delivered by John Spink at the Teagasc Weather Conference 2013, Teagasc Food Research Centre, Ashtown, Dublin, Ireland.